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CONTENTS

	Page
Support for Science Teaching in Schools	1037
Isotopes in the Service of Geology. By Prof. Arthur Holmes, F.R.S.	1038
Physical Measurement in Gas Dynamics and Combustion. By Dr. A. G. Gaydon, F.R.S.	1040
Malaria in Retrospect. By Dr. E. Ashworth Underwood	1040
Protein Synthesis in Isolated Cell Nuclei. By Dr. V. G. Allfrey, Dr. A. E. Mirsky and Syozo Osawa	1042
The Venezuelan Institute for Neurology and Brain Research. By Dr. Geoffrey H. Bourne	1049
Obituaries :	
Dr. J. W. Nicholson, F.R.S. By Prof. Wm. Wilson, F.R.S.	1051
Mr. Alexander Keiller. By Prof. Stuart Piggott	1051
Dr. E. J. Delporte. By Dr. J. G. Porter	1052
Prof. Robert P. Bigelow	1052
Nobel Prizes for 1955	1053
News and Views	1054
Conference of Commonwealth Survey Officers. By Brig. G. Bomford, O.B.E.	1057
International Biometric Symposium in Brazil. By Dr. D. J. Finney	1058
Design of Electronic Devices for Production. By J. Thomson	1059
Animal Movement	1060
Standards in British Industry	1061
The Polypeptide Chain Configuration of Collagen. By Drs. Pauline M. Cowan, Stewart McGavin and A. C. T. North	1062
Quantitative Separation of B Vitamins by Electrophoresis on Agar Plates. By Dr. G. Marten	1064
Valency Changes of Molybdenum during the Enzymatic Reduction of Nitrate in *Neurospora*. By Dr. D. J. D. Nicholas and Dr. H. M. Stevens	1066
Letters to the Editors :	
Function of Krause's Membrane.—A. F. Huxley, F.R.S., and Dr. R. E. Taylor	1068
Reaction of Starfish Spermatozoa to Histidine and Certain Other Substances considered in Relation to Zinc.—T. Fujii, S. Utida and T. Mizuno	1068
Role of Photochemical Processes in the Reduction of Nitrate by Green Algae.—Dr. Erich Kessler	1069
Respiratory Increase and Phosphorus and Nitrogen Metabolism in Sweet Potato infected with Black Rot.—Takashi Akazawa and Dr. Ikuzo Uritani	1071
Molecular Weight of Insulin in N,N-Dimethylformamide.—Dr. E. Douglas Rees and Dr. S. J. Singer	1072
Inhibition of Complement Activity by Di-*isopropyl* Fluorophosphate.—Dr. Elmer L. Becker	1073
Occurrence of an L(+)-Alanine-Dehydrogenase in *Bacillus subtilis*.—J. M. Wiame and A. Piérard	1073
Action of Ribonuclease on Living Cells *in vitro* and Synthesis of Deoxyribonucleic Acid.—H. Firket, S. Chèvremont-Comhaire and Prof. M. Chèvremont	1075
A Critical Method of measuring the Diameter of Living Bacteria with the Interference Microscope.—K. F. A. Ross	1076
Taxonomic Position of the Genus *Selenomonas* (von Prowazek).—M. H. Jeynes	1077
Paper Chromatographic and Biological Properties of Reserpine and Related Compounds.—Dr. R. J. Boscott and Dr. Amiya B. Kar	1077
A General Method for locating the Spots on a Paper Chromatogram of Tertiary Alkyl Phosphates, Thiolophosphates and Thionophosphates.—Dr. Irmgard K. H. Otter	1078
Growth of Pre-Breakdown Ionization Currents in Hydrogen.—Dr. R. W. Crompton, Dr. J. Dutton and Dr. S. C. Haydon	1079
Measurements of Retarding Potential on the Hollow Cathode.—Dr. A. W. Bright and J. S. Thorp	1079
Electrophoretic Measurement of the True Zeta-Potential of Arsenious Sulphide Sol in the Region of Slow Coagulation.—Prof. B. N. Ghosh and K. C. Ray	1080
Preparation of Aluminium Borohydride and its Applications in Organic Reductions.—J. Kollonitsch and O. Fuchs	1081
Structure of Sterculic Acid.—J. P. Verma, Bhola Nath and Dr. J. S. Aggarwal	1082
Humidity and Corrosion.—H. R. Ambler	1082

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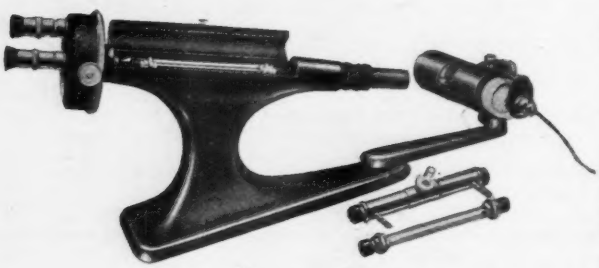

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SUPPORT FOR SCIENCE TEACHING IN SCHOOLS

ALTHOUGH the Appleton Committee, in reporting on the supply of teachers of mathematics and science in Scotland, rejected the suggestion that poor conditions in many school laboratories is a reason for the lack of recruits to science teaching, it did not deny that some school buildings are old and unattractive and that some laboratories are sparingly equipped. Moreover, if the standard of school science buildings and equipment is, in general, as satisfactory in England and Wales as the Appleton Committee found it to be in Scotland, the indications are that the standard is likely to deteriorate rapidly in the absence of special assistance. The maintained secondary schools are able to look to public funds to enable them to extend their laboratories and to improve their equipment and facilities for science teaching; and Sir David Eccles has said enough in recent months to justify them in facing the impending 'bulge' with some confidence that their resources will be increased sufficiently to enable them to cater efficiently for the anticipated and desired increase in scholars studying science.

The independent schools in Britain have no such confidence, and any expansion must be financed out of their own resources. They are reluctant to raise their fees further, although these have not risen enough to cover the whole cost of running and expanding the schools. If indeed fees have not yet reached the limit, that limit must shortly be set by the same factor of increasing taxation in the higher levels which is already drying up the private benefactions and endowments which in the past brought the schools into being, and have made possible the outstanding education for which they are famous.

These schools, with their fine tradition of scholarship in the humanities, represent a valuable potential source of scientists and technologists. But first it will be necessary to overcome the reluctance apparent in them to enter on such careers; a reluctance to which the Advisory Council on Scientific Policy and speakers in both Houses of Parliament in recent debates on scientific and technical man-power, the shortage of science teachers and similar themes have directed attention, as well as Sir Alexander Todd in his address to the Parliamentary and Scientific Committee last July on the scientific man-power problem. For that reason alone it is fitting that industry should come forward and assume some of the responsibility which private individuals are increasingly unable to discharge. The Industrial Fund for the Advancement of Scientific Education in Schools, which as announced in *Nature* of November 5, p. 860, has been established by seventeen chemical, electrical and mechanical engineering companies, is intended to provide funds for the considerable expansion in the provision of buildings and equipment which is required if the independent schools and direct grant schools in the United Kingdom, the Channel Islands and the Isle of Man are to make their fitting contribution to the increased supply of scientists and technologists that the nation needs.

This initiative was warmly welcomed by the Minister of Education, Sir David Eccles, in the course of a reply to a question in the House of Commons on November 1 regarding the improvement of facilities for advanced science teaching in the schools. In that reply Sir David said that since 1945, about a thousand new laboratories had been built or started in maintained grammar or technical schools. In a written reply on November 10 he estimated the cost of these as £1·5 million in technical schools, just under £3 million in grammar schools and £9 million in modern secondary schools. He expressed the hope that authorities will continue to use their freedom in minor works to improve science accommodation; but he said he is ready to consider on their merits projects which could not be executed for less than £10,000.

Whatever reservations may be held as to the effect of the establishment of the Industrial Fund for the Advancement of Scientific Education in Schools on the supply of science teachers, it cannot be other than beneficial and will enable the schools to offer opportunities in science to an increasing number of boys and girls. This is desirable not only from the point of view of public or industrial administration, but also from that of the production of scientists or technologists, as Sir Henry Tizard emphasized in his Haldane Memorial Lecture last March. Moreover, it should be noted that the examples of research carried out in schools cited by Dr. A. G. Lowndes in his paper at the Birmingham meeting of the British Association were carried out in independent schools. The improved facilities which this new Fund may make possible in such schools should assist the recruitment of first-class science teachers, and Mr. J. T. Stock has recently directed attention to this aspect. In a recent note in *The Times Educational Supplement*, he described the results which have followed the granting to him and a colleague at Norwood Technical College, in consequence of the Ministry of Education Circular 94 of 1946 on Research in Technical Colleges, of five hours a week relaxation of teaching duties for research in chemistry. Some 90 per cent of this work, largely on the design and development of micro- and semi-micro-apparatus and techniques, has been carried out with equipment and materials normally available in schools.

This particular work has received a fair amount of recognition, notably in exhibits at the Science Museum and at the International Congress on Analytical Chemistry at Oxford in 1952 and in a B.B.C. Science Review telefilm; but its importance in the present connexion is its demonstration of the potentialities of research with a modest official allowance of time. The factor is certainly one not to be discounted lightly; and it is even possible that,

as regards the recruitment of science teachers and the supply of scientists and technologists, the great value of the establishment of the new Fund will lie in the increased prestige which is thereby given to science and to technology as careers. Indeed, if its full effect is to be felt, it is important that scientists should make every effort to overcome whatever obstacles continue to hinder the ablest boys and girls in the independent or the grant-aided schools from taking up a science career. Equally, of course, it is important that other firms should follow the lead of the institutors of the Fund.

In this connexion it is worth recalling the provocative remark made by Sir Henry Tizard thirty-five years ago, and which he quoted in his Haldane Memorial Lecture, that the fame of the old School of Greats at Oxford was not really due to the intrinsic merits of the two subjects studied, but rather to the fact that men who took high honours in it had been specially selected at school to read classics because of their outstanding ability. Sir Henry went on to argue that any other two subjects would serve equally well for the training of the minds of men who are going into the Civil Service and other professions, provided that they are equally difficult and well taught, and to suggest that in this scientific age one of the subjects of Modern Greats should be science, and the other modern philosophy, history or economics. The suggestion was even then taken quite seriously; but its relevance to the teaching of science in the independent schools is in stressing the importance of quality. If, as there seems to be some reason for believing, in spite of the increase in the number of university students since the War, there has been no proportionate increase in the number of those obtaining first-class honours, it is of the utmost national importance that a reasonable proportion of the ablest students entering the universities each year should study science.

To ensure this, there must be no undue bias against science in the schools; and every effort must be made to see that the opportunities of science and technology as careers are fairly presented, and that the claims of science and technology as intellectual disciplines are fully understood by headmasters and their staffs. On them must rest a large responsibility for guiding the ablest young minds under their care to consider the claims of a career in science or technology; but it is no less important that such careers should have the prestige and status in the public mind which can be as important as remuneration in attracting recruits of high quality. The decisive factor is often the influence of the science master himself. If the new Fund, by furthering the provision of laboratories and equipment and improving teaching conditions generally, increases the prestige of science and those who teach it in the schools, then it may well help to attract teachers of ability and enthusiasm. In this way it may make a decisive contribution, not so much to the early removal of the shortage of science teachers, as to ensuring that a fitting proportion of the ablest minds in the sixth forms of schools each year turn to science or technology as a career.

ISOTOPES IN THE SERVICE OF GEOLOGY

Isotope Geology

By Prof. Kalervo Rankama. Pp. xvi+535. (London: Pergamon Press, Ltd.; New York: McGraw-Hill Book Company, Inc., 1954.) 75*s.* net.

IN 1950 Prof. K. Rankama proposed the term 'isotope geology' to cover the investigation of geological problems by means of research on the occurrence and natural history of isotopes, and especially the variations of their relative abundances. Some of the vast possibilities of this fascinating and rapidly expanding field of discovery are being actively explored by several enthusiastic teams of workers, and already the output of literature has become an accelerating stream with which only a specialist can hope to keep pace. Geologists have therefore good reason to be grateful to Prof. Rankama for giving them this admirable account of the principles of the subject and for making so readily available the results achieved up to very nearly the date of publication. The book is divided into two parts, of which the first is a remarkably lucid account of the physical and chemical principles involved in the various methods and techniques, while the second is devoted to summarizing and discussing all the relevant information about the isotopes of most of the elements, with special reference to actual examples of isotope fractionation in Nature and the geochemical processes responsible. Part 2 is much more than a compilation of data and a guide to the literature—although in these respects alone it is invaluable—for on every appropriate occasion the author discusses the geological significance of the results and directs attention to the many critical gaps where more data are urgently required. Indeed, few books can have been written in which so many opportunities for productive research are freely presented. For example, we can now clearly see how to solve some of the more tantalizing problems of petrogenesis and ore deposits, while at the same time realizing that each successful forward step will require organized and patient team-work, and the expenditure of much time and money. The stimulating effects of this book should help to shorten the time very substantially by adding to the numbers of eager workers.

Among the problems to which useful contributions have already been made, the measurement of geological time stands out as by far the most familiar and successful. During recent years the radiocarbon method has made it practicable to begin the construction of a world-wide 'atomic calendar', going back perhaps some thirty thousand years, which would provide an 'absolute' chronology if the fundamental assumption of long-term constancy of cosmic-ray intensity could be justified. Dating of samples already dated by varves is the obvious requirement; but as yet this has not been systematically carried out. Indeed, if E. Antevs (*J. Geol.*, **63**, 499; 1955) is correct in concluding that the radiocarbon age for the celebrated Two Creeks forest bed (11,400 years) is at least 8,000 years too young, the method will eventually disprove the assumption and show how the intensity of cosmic rays can be 'calibrated' over the period that has been measured by varves. At the other end of the known time-scale, the methods based on the accumulation of radiogenic isotopes of lead have been successfully applied to the dating of meteorites, by the use of techniques that would have

seemed little short of miraculous a few years ago. All the many topics involved in geochronology—and a few outside, such as the 'age' of the elements themselves—are treated competently and critically, with a firm and frank assessment of the difficulties that have still to be overcome. For example, in discussing whether or not the rates of decay of the radioactive nuclides have varied with time, Rankama pertinently points out that the argument for constancy based on measurements of pleochroic haloes is not infallible, since a considerable variation in the decay constant would be necessary to produce a detectable change in the range of the alpha-particle concerned.

In the field of petrogenesis the author very generously gives me credit for having made the first constructive suggestions involving 'isotope geology', based on the inference that the isotope of calcium generated by the radioactive isotope of potassium would increase during geological time about twenty-seven times as fast in granites as in basaltic rocks. At that time (1932) it seemed to be quite practicable to determine the abundance-ratios that were needed to decide whether various rocks had been formed by differentiation or not, and if not, whether some such process as replacement or transfusion had operated. This optimism was based on the mistaken belief, then current, that the radioactive isotope of potassium was of mass 41 and that its decay product was calcium-41. It was not until 1935 that Nier showed the active nuclide to be potassium-40. In the interim, as Rankama records on p. 315, "the method was actively investigated by the magneto-optic method developed by F. Allison in the United States, but no results were published". It was very fortunate for us that we were cautious enough to abstain from publishing, and it may be of interest to place on record how we eventually came to realize that, whatever was being measured, it was not calcium-41. Over a period of two years I sent scores of analysed rock-powders to Dr. Allison for the determination of 'calcium-41'. The results were dramatically exciting and, despite a few disturbing discrepancies, I felt sure that we had found a powerful new key for unlocking at least some of the secrets of petrogenesis. In the hope of tracing the source of the discrepancies I sent a dozen samples of marine limestones, fossils and shells of known age, ranging from the present to far back in the Pre-Cambrian, expecting to find that the ratio of 'calcium-41' to total calcium had increased systematically during geological time. Alas, there was no increase! The Pre-Cambrian ratios did not differ appreciably from the values for modern shells and sea water. This pulled us up with a painful jerk; and the discovery about the same time that no such isotope as calcium-41 is detectable by the mass spectrograph (the decay product in question being calcium-40) raised what were then, and still are, insuperable difficulties against making a fresh start with the right isotopes. Increase of the ratio of strontium-87 to strontium-86 with geological time should be much more easily detectable in marine chemical sediments, as Wickman pointed out in 1948; but Rankama has still to report that even this highly promising method has so far failed to yield satisfactory results.

More positive progress in tracing the origins of rocks, minerals, natural waters and gases has been achieved by determining the variations in the isotopic constitutions of such elements as carbon and oxygen. The chapters on these elements are consequently among the longest and most informative. Baertschi's investigations are notable for their demonstration that both oxygen-18 and carbon-13 vary considerably in metamorphic limestones, ranging from the relatively low values characteristic of sedimentary types to the higher values given by carbonatites of igneous origin. The high values occur at contacts where, presumably, great quantities of juvenile carbon dioxide, water, etc., traversed the rocks and effected isotope exchanges during their passage. Various investigators have found that oxygen-18 tends to be more abundant in granitic than in basaltic rocks, which is not what would be expected if these rocks were related by differentiation. The heavy isotope of silicon also tends to concentrate slightly in the more silicic rocks. However, much more evidence of this kind is required for a variety of elements, in critically selected suites of rocks having known relationships, before firm conclusions can be reached, and here the co-operation of field geologists is essential. Rankama's chapter on magnesium—twenty lines, only—reflects a paucity of data for a very common element that is lamentable in view of the many problems connected with stony meteorites, ultrabasic rocks and basalts that still await solution.

Important applications that are already under way include the assessment of the palæo-temperatures corresponding to the growth conditions of carbonate shells of Jurassic and later ages (the 'carbonate thermometer') and of the distributions of climate so implied. Similarly, the isotopic constitution of sulphur in pyrite depends on the temperature at which the mineral crystallized; for example, in hydrothermal deposits the crystals become relatively enriched in sulphur-32 as the temperature decreases. A study of marine sulphates and sulphides of various ages has suggested to Thode and others that organic fractionation of sulphur did not occur on an appreciable scale until towards the close of the Pre-Cambrian. Here there is a hint that the earlier atmosphere may have been deficient in free oxygen and that the later rapid development of oxidizing conditions may have favoured the extraordinary efflorescence of Cambrian life-forms. While sounding a cautious note of warning, Rankama comments on the fascination of these problems and the urgent need for much more detailed research.

The book has a bibliography of more than thirteen hundred items, an index of authors and an unusually detailed and efficient subject index. The only criticism as to style is that the author has picked up the bad habit of using the word 'data' as if it were a singular noun. It may not be out of place to direct the attention of publishers' readers to this all-too-common fault. Few misprints have been detected; one that should perhaps be mentioned occurs in equation 75.27 on p. 383, where ^{235}U should be ^{238}U. There is a related mistake in the reference to me on p. 386, concerning which I need only say that I was not guilty of having paid no attention to the isotopic constitution of uranium in my calculations.

Prof. Rankama is to be congratulated on a very impressive achievement, and the publishers on the production of a handsome volume that is worthy of its contents. The book will be found invaluable by scientific workers in a wide variety of subjects besides geology and geochemistry, and many of its readers will doubtless join with the reviewer in the hope that supplements embodying the results of current and future researches in the same fields may be prepared at intervals by this distinguished author to whom we owe so much already.

ARTHUR HOLMES

PHYSICAL MEASUREMENT IN GAS DYNAMICS AND COMBUSTION

Physical Measurements in Gas Dynamics and Combustion

Edited by R. W. Ladenburg, B. Lewis, R. N. Pease and H. S. Taylor. (High Speed Aerodynamics and Jet Propulsion—Vol. 9.) Pp. xvi+578. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1955.) 84*s*. net.

THE great advances in aeronautical science have been accompanied by remarkably fast developments in experimental techniques for studying the various aspects of the flow of gases. The first part of this volume, which is the ninth of a series of twelve on high-speed aerodynamics and jet propulsion, deals with methods of measurement of density, pressure, temperature, velocity and state of turbulence. Developments in the usual methods, such as schlieren photography, interferometry, hot-wire anemometry, piezoelectric pressure gauges, temperature measurements from infra-red radiation, and shielded thermocouples, are described. A number of interesting, and sometimes surprising, recent or possible techniques are also examined: these include the use of active nitrogen for following density changes at very low pressure; the use of ultra-violet or X-ray absorption spectra to measure density; determination of velocity from the quenching of glow discharges; study of polarization and wave-length distribution of scattered light for mist condensation problems; the use of light reflected at grazing incidence to determine the thickness of a shock wave; and the determination of the surface temperature of projectiles in flight by coating them with a temperature-sensitive phosphor.

The second part of the book deals with measurements on combustion gases; this is restricted to physical methods, as another volume, on "Combustion Processes", is in preparation. There are chapters dealing with flame photography, temperature measurements, determination of burning velocity, flame spectroscopy, the use of mass spectra, and analysis of the structure of the flame front. Standard methods are again covered, and some new techniques are discussed; these include developments in the spectrum-line reversal method of temperature measurement to give the temperature of complex flames, and the use of line broadening and Doppler shift to give pressure and gas velocity in rocket-motor flames; spectroscopic determination of effective rotational temperature; and the study of the thick reaction zone of flames at very low pressure.

The sections in the part of the book on gas dynamics tend to be rather short and specialized, so that the effect is like a series of articles in a review journal, such as *Reviews of Modern Physics*, rather than a unified book. The combustion section, although limited to physical measurements, consists of longer, more comprehensive articles. Dr. E. F. Fiock's on burning velocities is very clear and includes new results; Dr. F. P. Bundy and Dr. H. M. Strong's is particularly good on the spectroscopic temperature measurements; Dr. W. T. Reid's on flame photography is easy to read but incomplete; Prof. G. H. Dieke gives a good account of developments in photoelectric spectroscopy as applied to flames; and Mr. N. Thomas stresses the value of the new techniques for the detailed understanding of flame structure.

The rapid developments in the many aspects of this field of research made the book necessary. The inevitable time-lag in publication is probably responsible for some omissions; there does not appear to be any mention of quantitative neutral-wedge schlieren photography, or of studies of flame structure from measurements of refractive index made with an inclined slit.

The book comprises twenty-four articles by twenty-two authors. Generally the standard is high, and these articles, at an advanced level, in which so many developments in techniques are discussed, are welcome. Most of the articles are naturally rather specialized, so that most people working in some part of this extensive field will find a few articles of considerable value but also others which cannot easily be read. There is a single collected subject-index for the whole book, but no author-index. The full references at the end of each article are, of course, valuable.

A. G. Gaydon

MALARIA IN RETROSPECT

Man's Mastery of Malaria

By Dr. Paul F. Russell. (University of London, Heath Clark Lectures 1953.) Pp. xv+308+24 plates. (London: Oxford University Press, 1955.) 25*s*. net.

THE sixteen years which have elapsed since the start of the Second World War have seen great developments in the control and treatment of malaria. In Ceylon the case-rate per 1,000 living has fallen from 574 in 1940 to 11 in 1953. In Greece the total deaths from malaria in 1942 were 1,856; in 1950 there were only 14 deaths from this disease. In Italy, Sicily and Sardinia malaria has been practically eradicated. An illuminating commentary on improvements in treatment is given by the fact that, whereas in Italy, Sicily and Sardinia approximately 300,000 cases resulted in 8,407 deaths in 1919, in 1945 approximately 400,000 cases resulted in only 386 deaths. Since 1948 there have been no malarial deaths in the Italian Republic. Results of this nature are conducive to a sanguine attitude, and an expert assessment of the position is therefore to be welcomed. Dr. Paul F. Russell devoted his Heath Clark Lectures to this subject in 1953, and in their published form they provide an excellent résumé of the history of the disease and an authoritative discussion of the results of recent measures.

Malaria has a venerable history. Empedocles is supposed to have delivered the city of Selinus from pestilence by diverting two neighbouring streams so that they cleansed the mouth of the river. In Roman times malaria was associated with marshes, and in the Campagna and elsewhere Roman engineers carried out much drainage. Hippocrates gave excellent descriptions of the clinical features of malaria, and also commented on the effect of marshes and stagnant waters in producing enlarged spleens. Despite the warnings given by Celsus, Varro, Columella, Galen and other classical authors, the Pontine Marshes were not successfully drained until the ten-year task which ended in 1940, and successful drainage in Greece is even more recent.

In the case of the malaria parasite there was also an unconscionable lapse of time between the first glimmerings of recognition and the period when practical use could be made of the discovery. The characteristic pigment was seen in spleen and brain

sections by Lancisi in 1716 and by Bright in 1831. They did not associate the pigment with malaria. Both Virchow and Frerichs described the pigmented cells and associated them with malaria. Frerichs and Delafield (1872) probably described the parasite—the plasmodium—without recognizing it. In 1880 Alphonse Laveran recognized the fact that these bodies were living parasites, and as a result of his brilliant studies and the work of the Italian investigators, Marchiafava, Celli, Golgi and their collaborators, part of the life-history of the vivax and quartan parasites was known within the next few years. Yet in 1886 Osler was definitely sceptical—until he had found the crescent forms for himself. During all this period there was much speculation on the means whereby the parasite was transferred from an infected to an uninfected individual. Mosquitoes had been suspected from ancient times, but Beauperthuy (1854) was probably the first medical writer to state that they were vectors. In 1882 King, of Washington, D.C., listed nineteen reasons why mosquitoes must be considered responsible, and he was so convinced of his rightness that he recommended that the whole city of Washington should be invested in a closely woven metal screen as high as the Washington Monument. Manson's brilliant mosquito–malaria hypothesis (1894) is well known; but it is perhaps not so frequently realized that he missed the fact that the parasite, sucked from man by the mosquito, was later injected directly into another individual. He thought that the spores of the malaria parasite were liberated by the mosquito into water, which was later drunk by the individual who became infected. Ross devoted three years of intensive research to the discovery of the malaria sporozoites in the salivary gland of a mosquito, and although he worked out fully the life-cycle of the malaria parasite of birds (proteosoma), by ill-luck he was denied the final proof of the man-to-mosquito-to-man cycle, which was made by Italian workers in 1898.

For nearly fifty years after these crucial discoveries there was doubt regarding what actually happened after an infected mosquito bit an uninfected individual. In 1903 Schaudinn described how the sporozoites penetrated the human red-blood corpuscles; but no one else had ever seen this phenomenon. It was not until just before the Second World War that it was discovered in birds that the sporozoites developed into pre-erythrocytic forms in the liver, spleen and bone-marrow, and that these forms then developed into unpigmented exoerythrocytic forms before they invaded the blood corpuscles. The demonstration of these phenomena in the human subject demanded a healthy individual who was willing to be infected experimentally and then to have a small portion of his liver removed for histological examination. In 1948 and 1949 this important demonstration was made for *Plasmodium vivax* and *P. falciparum* by Shortt and his colleagues. The incubation period was thus explained, and a better starting-point was provided for the testing of new therapeutic remedies.

Much has been written on the history of cinchona from its introduction to Europe in 1632. Pelletier and Caventou discovered quinine and cinchonine, two of its four basic alkaloids, in 1820, and the other two, quinidine and cinchonidine, were discovered within the next twenty-five years. Although obviously specifics for malarial attacks, all these substances have certain disadvantages. Some of these have been obviated by the newer synthetic drugs, such as pamaquine, mepacrine, chloroquine, proguanil and daraprim. Despite the great strides which have been made, the ideal drug for both prophylaxis and treatment has probably still to be synthesized.

In this sphere of prophylaxis, enormous progress has been made by destruction of mosquito larvæ and by preventing bites of adult insects. In the destruction of larvæ, oil was first used by Dunlap in 1793, and paris green was introduced in 1916. The work of Gorgas during the cutting of the Panama Canal was a magnificent example of what could be done by persevering work of this nature. In the campaign against the adult mosquito, DDT was a discovery of the highest practical value.

In these valuable lectures Dr. Russell has presented a very detailed summary of the history of malaria in language which can be understood by anyone who knows the barest elements of protozoology. He avoids the clinical features of the disease and concentrates on the parasite, the mosquito, and the results of prophylaxis and treatment. The word 'summary' is used advisedly. The literature of the subject is enormous, and Dr. Russell's very extensive bibliography is merely a token of what is available. It need scarcely be said that it is very carefully selected. Though Dr. Russell is perhaps not quite at home with the older material, he is a complete master of the modern literature. It is surprising that the Oxford University Press has allowed a number of un-English terms to slip through—such as 'editorialized' and 'sanitating'; after all, the lectures were delivered in one English university and printed at the press of another. It is disconcerting also to find in the text that the title of an article in a journal is italicized as though it were a book—though the conventional practice is always used in the bibliography. Otherwise, the production of the book comes up to the high standard of previous Heath Clark Lectures.

In his preface Dr. Russell specifically explains that the title of his book does not necessarily mean that malaria has been eradicated. There are, indeed, some small clouds on the distant horizon. Although malaria parasites were exposed to cinchona and its alkaloids for three hundred years without showing signs of drug resistance, some strains have now begun to show this property. Further, in some areas these strains have been spreading—for example, *Plasmodium falciparum* in Malaya and *P. vivax* and *P. malariae* in Indonesia. At present this resistance can be overcome by the administration of full doses of proguanil from the outset; but the parasite apparently has the next move. Again, certain species of mosquitoes have become resistant to DDT, so that in Greece and Java this method of prophylaxis is no longer possible. There are many sociologists and demographers who hold that the great advances made in the control of malaria will lead to epidemics of that disease, or alternatively to over-population and famine. In the final part of his book Dr. Russell skates gracefully over this slippery ice without attempting to make much headway. Perhaps he does not intend to, since the legitimate goal of the medical man is yet far distant. Dr. Russell estimates that at the present time 250 million persons suffer attacks of malaria yearly and 2·5 million die of it. In this field there is still sufficient work in hand for the Rockefeller Foundation, of the staff of which Dr. Russell is a distinguished member.

E. Ashworth Underwood

PROTEIN SYNTHESIS IN ISOLATED CELL NUCLEI

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IN experiments described previously, it was noted that nuclei prepared from the thymocytes of the calf can actively incorporate DL-alanine-1-^{14}C into their proteins when incubated *in vitro*. This activity of the isolated nucleus required that its deoxyribonucleic acid remain intact: the incorporation of amino-acid was seriously impaired by pretreatment of the nucleus with deoxyribonuclease. A partial recovery of deoxyribonuclease-treated nuclei could be observed when a supplement of calf thymus deoxyribonucleic acid was added to the incubation mixture[1].

The present communication is concerned with the further investigation of protein synthesis by the isolated nucleus, with the role of deoxyribonucleic acid (DNA) in this process, and with the capacity of the thymocyte nucleus to meet its energy requirements through the synthesis of high-energy phosphate bonds.

A rapid procedure for the preparation of nuclei from the thymus of the calf has already been described[1,2]. In its essentials, the method involves a controlled homogenization of the tissue in cold 0·25 *M* sucrose solution containing a small amount of calcium chloride. Coarse fibre is removed by filtration through gauze and through flannelette, and the nuclei are separated by fractional centrifugation and thoroughly washed. In the experiments about to be described the nuclei were prepared in 0·25 *M* sucrose containing 0·0020 *M* calcium chloride.

Nuclei so isolated meet a surprising number of the requirements which we consider desirable and necessary before a study of their biochemical attributes is undertaken. First, in overall composition and in enzymic constitution these 'sucrose' nuclei are the equivalent of nuclei prepared from the thymus under non-aqueous isolation conditions, that is, by a modified Behrens' procedure[3,4]. The latter nuclei are selected as a standard of comparison because their purity has been established by chemical, enzymatic and immunological tests, and because only non-aqueous conditions of isolation preclude an exchange of water-soluble materials between nucleus and cytoplasm. A detailed comparison of thymus nuclei isolated in sucrose with those prepared by the Behrens' procedure has shown that nuclei isolated in sucrose have retained their water-soluble enzymes[2]. The deoxyribonucleic acid content is the same (2·5 per cent DNA-P) for nuclei prepared by the two procedures, which indicates that their overall protein content is also the same.

Other evidence concerning the integrity and cleanliness of the 'sucrose' nuclei comes from careful examination of such preparations under the microscope. We have made use of ordinary light, phase-contrast, interference and electron microscopy. The electron microscope is of particular value for discerning and estimating the extent of contamination by intact, small thymocytes. The proportion of such intact cells in nuclear preparations is relatively low. For example, two preparations examined in this way have shown 45 and 77 small thymocytes per thousand nuclei, respectively. Thus the electron micrographs support the demonstration by other optical and chemical methods that there is no gross contamination of the nuclei with whole cells or cytoplasmic debris[5]. However, it should be noted that careful microscopic examination often reveals small bits or strands of cytoplasm adhering to some of the nuclei. The extent of this contamination cannot be estimated quantitatively under the microscope, but nucleic acid analyses and enzyme measurements make it clear that the level of cytoplasmic contamination lies well below 10 per cent.

When a suspension of nuclei in buffered sucrose medium is shaken at 37° C., in the presence of DL-alanine-1-^{14}C or glycine-1-^{14}C, there is a rapid incorporation of the isotope into nuclear proteins. It should be mentioned that what is actually measured in most of the experiments described is the average specific activity of the proteins which are precipitated in 5 per cent trichloroacetic acid (TCA). Nucleic acids are extracted and removed with hot trichloroacetic acid and lipides are removed by successive treatments with hot alcohol, alcohol–chloroform–ether mixtures, ether, and acetone. The remaining protein is homogenized in acetone, collected on filter-paper planchets, and the carbon-14 activity measured using a Geiger–Müller tube and scaling circuit[1]. More than 94 per cent of the carbon-14 activity of the isolated protein remains after treatment with 0·25 *N* sodium hydroxide for two hours, and more than 89 per cent remains after reaction with ninhydrin.

The time course of a typical incorporation experiment is given in Fig. 1. It can be seen that after an initial lag period of 10–15 min., ^{14}C-amino-acid incorporation proceeds linearly up to 90 min. and then begins to taper off. The phenomenon is aerobic. The incorporation of ^{14}C-alanine or ^{14}C-glycine in an atmosphere of nitrogen is negligible (Table 1). The uptake is also greatly inhibited by 10^{-3} *M* sodium cyanide and by 10^{-4} *M* dinitrophenol. Methylene blue ($2{\cdot}5 \times 10^{-4}$ *M*) has no effect on amino-acid incorporation. The addition of a supplementary energy source leads to a slight increase in nuclear activity. For example, glucose or α-ketoglutarate added to the incubation medium raises the level of isotope incorporation by 10–20 per cent.

One of the most striking effects on nuclear amino-acid incorporation is that produced by sodium ions. Nuclei isolated by the procedure described require a sodium ion supplement to the medium in order to synthesize protein actively. The effect of varied sodium concentration on the level of amino-acid uptake is shown in Fig. 2. There is a well-defined maximum at about 0·068 *M*. The specificity of the sodium require-

* Fellow of the Rockefeller Foundation.

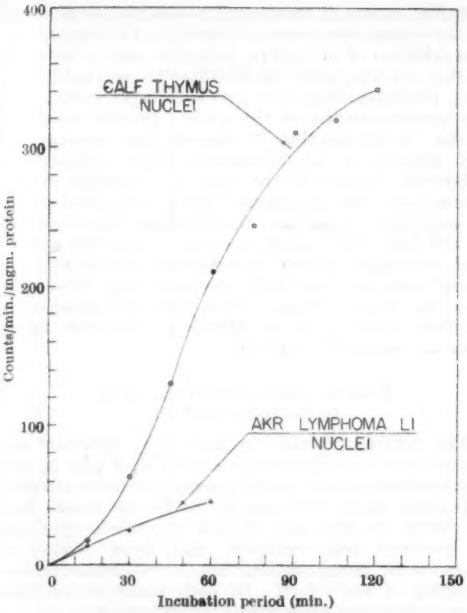

Fig. 1. The time course of incorporation of alanine-1-^{14}C into the proteins of isolated nuclei. The incubation flasks contained 1 ml. of nuclear suspension (100 mgm. (wet weight) of nuclei per ml. of 0·25 *M* sucrose – 0·0020 *M* $CaCl_2$), 0·5 ml. of 0·1 *M* sodium phosphate – 0·25 *M* sucrose buffer at *p*H 7·3, 0·4 ml. of 0·1 *M* glucose containing 6·25 mgm. of NaCl per ml. and 0·1 ml. of DL-alanine-1-^{14}C solution containing 0·5 mgm. = 0·008 mc. per ml. The flasks were shaken in a 37° bath for the indicated periods and the uptake then halted by the addition of an equal volume of 10 per cent trichloracetic acid. The protein was prepared and its carbon-14 activity determined as previously described (ref. 1)

ment is emphasized by a series of experiments summarized in Fig. 3. In these tests an attempt was made to substitute all or part of the sodium in the incubation medium with an equal amount of potassium. In all cases the total salt concentration was kept constant. It is evident that when all the sodium is replaced by potassium the synthetic activity falls to 15 per cent of the optimal value; increasing the Na^+/K^+ ratio results in a corresponding increase in the amount of amino-acid incorporated. The extreme dependence of the synthetic activity of the nucleus upon the sodium ion concentration has made it necessary to control rigorously the sodium-level of the incubation medium. In all the experiments described below supplements of sodium chloride were added to bring the final sodium ion concentration to 0·068 *M*. The relationship between the sodium- and potassium-level in the medium and that within the nucleus is at present being investigated.

Table 1. Effect of Anoxia, Cyanide, Dinitrophenol and Added Metabolites upon ^{14}C-labelled Amino-acid Incorporation by Isolated Calf Thymus Nuclei

Conditions of experiment	Isotope administered as	Specific activity of nuclear protein (c.p.m./mgm.)
A. Nuclei in O_2	glycine-1-^{14}C	71
Nuclei in N_2	,,	14·7
B. Nuclei in O_2	DL-alanine-1-^{14}C	72
Nuclei in N_2	,,	10·4
C. Nuclei without added dinitrophenol	DL-alanine-1-^{14}C	72
Nuclei in 2 × 10^{-4} *M* dinitrophenol	,,	11
D. Nuclei without added dinitrophenol	DL-alanine-1-^{14}C	154
Nuclei in 1 × 10^{-4} *M* dinitrophenol	,,	58
E. Nuclei without added cyanide	DL-alanine-1-^{14}C	64
Nuclei in 0·001 *M* sodium cyanide	,,	13
F. Nuclei without added metabolite	glycine-1-^{14}C	86
Nuclei + 40 μM glucose	,,	99
Nuclei + 40 μM α-keto-glutarate	,,	109

The synthetic activity of the isolated nucleus can be readily destroyed either by heating, or by freezing and thawing. Nuclei stored at 2° C. gradually lose their ability to incorporate ^{14}C-amino-acids. The rate of decline in activity with storage varies considerably in different preparations; but in most cases activity is well retained for 4–6 hr. and almost entirely lost after 24 hr. at 2°. Nuclei prepared in hypertonic sucrose solutions (for example, 1 *M*) are inactive. Nuclei prepared in 0·25 *M* sucrose and afterwards placed in 1 *M* sucrose solution also lose their activity.

Nature of the Incorporation Process

There are several experiments now to be described which make it clear that the uptake of labelled amino-acids into nuclear proteins represents protein synthesis and is not simply a random or non-specific exchange reaction.

The first of these experiments is the demonstration that only the L-enantiomorph of the administered DL-alanine-1-^{14}C is incorporated into nuclear protein. The ability of the nucleus to distinguish between

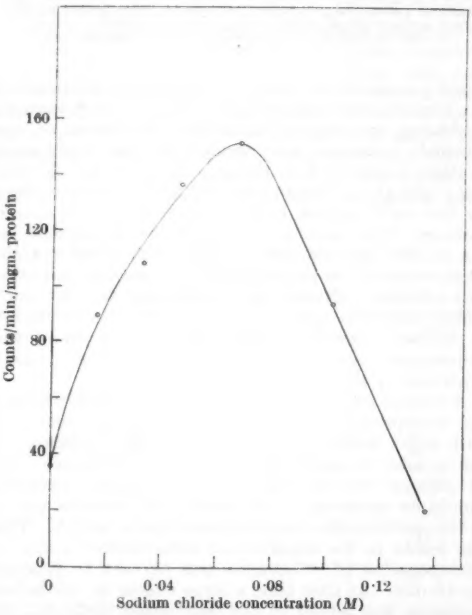

Fig. 2. Effect of sodium (chloride) concentration upon glycine-1-^{14}C incorporation by isolated thymus nuclei. The incubation flasks contained 1 ml. of nuclear suspension, 0·5 ml. of 0·03 *M* potassium phosphate – 0·175 *M* sucrose buffer at *p*H 7·3, 0·4 ml. of 0·1 *M* glucose containing varied amounts of sodium chloride, and 0·1 ml. of glycine-1-^{14}C solution containing 1·0 mgm. = 0·018 mc. per ml. The incubation period was 60 min.

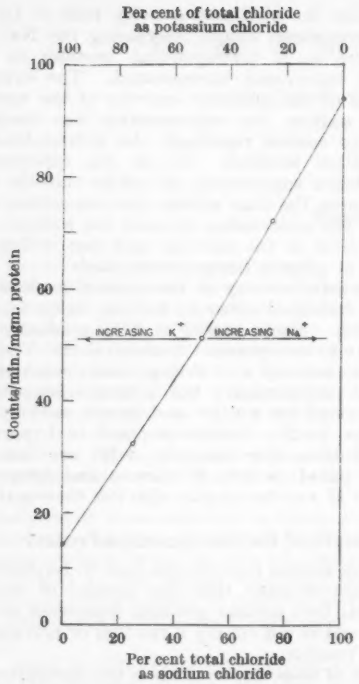
Per cent of total chloride as potassium chloride
100 80 60 40 20 0
Counts/min./mgm. protein
INCREASING K+
INCREASING Na+
Per cent total chloride as sodium chloride

Fig. 3. Effect of varying sodium : potassium ratio upon glycine-1-^{14}C incorporation by isolated thymus nuclei. The incubation flasks contained 1 ml. of nuclear suspension, 0·5 ml. of 0·03 *M* potassium phosphate – 0·175 *M* sucrose buffer at *p*H 7·3, 0·4 ml. of 0·1 *M* glucose containing 0·137 m*M* of total chlorides, the ratio of sodium to potassium being varied as indicated in the chart, and 0·1 ml. of glycine-1-^{14}C solution containing 1·0 mgm. = 0·018 mc. per ml. The incubation period was 60 min.

D and L forms of the amino-acid is readily indicated by the experiments summarized in Fig. 4. In these tests increasing amounts of unlabelled D-alanine or unlabelled L-alanine were added to the incubation medium together with the DL-alanine-1-^{14}C. If both the D and the L forms of the ^{14}C-alanine were utilized by the nucleus then both unlabelled isomers should compete with isotopic molecules for acceptance by the nucleus, and the carbon-14 activity of the nuclear protein should be correspondingly lowered. Actually, the addition of unlabelled D-alanine has no such effect ; only the L-isomer competes for incorporation. It follows, therefore, that only the L-form of the ^{14}C-alanine administered is utilized for protein synthesis by the nucleus.

A second experiment was designed to test whether the incorporated amino-acid is in constant exchange with other amino-acid molecules in the medium. If the uptake of amino-acids is indeed reversible then ^{14}C-alanine once incorporated into nuclear proteins should be exchanged with unlabelled L-alanine added to the medium after incorporation has occurred. This was tested in the experiments summarized in Fig. 5. Incorporation of ^{14}C-alanine was allowed to proceed for 45 min. At that time a large excess of unlabelled L-alanine was added to the incubation medium. In 'control' experiments an equal amount of unlabelled D-alanine was added. Samples of the nuclei were removed at 15-min. intervals and the carbon-14 activity of the nuclear protein measured in the usual way. It is evident from the curves that adding an eightfold excess of unlabelled D-alanine has no effect on the normal time course of carbon-14 incorporation. The addition of unlabelled L-alanine acts to suppress further incorporation of alanine-^{14}C, as could have been predicted from the previous experiment ; but the specific activity of the nuclear protein continues to rise. It follows that ^{14}C-alanine once incorporated into protein is not constantly being replaced by unlabelled alanine in the medium. Similar experiments have been performed using a 200-fold excess of unlabelled L-alanine. No exchange was noted up to 180 min. after addition of the unlabelled amino-acid, although 'control' experiments showed that the nuclei were still actively incorporating ^{14}C-alanine over the entire period. Thus the incorporation of labelled amino-acids represents a synthetic rather than an exchange reaction.

Role of Deoxyribonucleic Acid in Protein Synthesis

The notion originally suggested by Brachet[6] and Caspersson[7] that ribonucleic acids play a part in protein synthesis is now widely accepted as demonstrated. The most direct evidence in its favour comes from the work of Gale and Folkes on protein synthesis in bacterial cell residues[8], and from studies of amino-acid incorporation by a ribonucleoprotein complex of the liver[9]. In both cases ribonuclease acts to suppress amino-acid incorporation.

The isolated cell nucleus affords a unique opportunity to test the role of the deoxyribonucleic acids in the process of protein synthesis, and the results have a special interest because they ultimately bear on the mode of action of the gene, and on the chemical relationships between the nucleus and the cytoplasm.

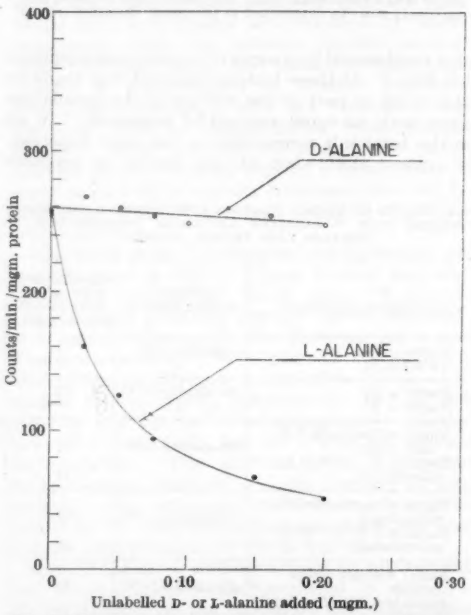

Fig. 4. The effect of unlabelled D- and L-alanines upon incorporation of DL-alanine-1-^{14}C by isolated thymus nuclei. The incubation vessels contained 1 ml. of nuclear suspension, 0·5 ml. of 0·1 *M* sodium phosphate – 0·25 *M* sucrose buffer (*p*H 7·3) containing varied amounts of either D- or L-alanine, as indicated in the chart, 0·4 ml. of 0·1 *M* glucose containing 6·25 mgm. NaCl per ml. and 0·1 ml. of DL-alanine-1-^{14}C solution containing 0·5 mgm. = 0·008 mc. per ml. The incubation period was 60 min.

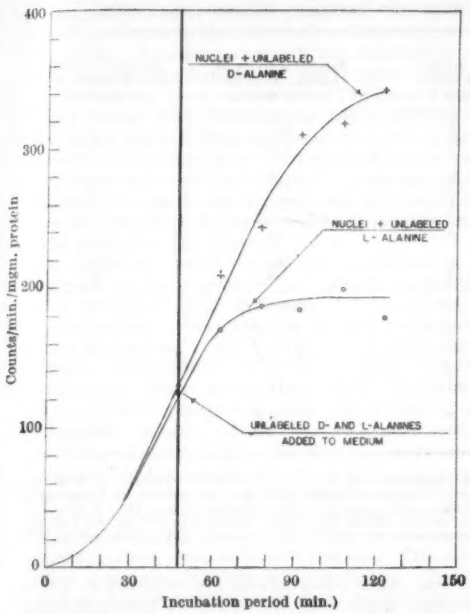

Fig. 5. The retention of incorporated alanine-1-^{14}C following addition of a large excess of unlabelled D- or L-alanine after uptake has occurred. The incubation vessels contained 1 ml. of nuclear suspension, 0·5 ml. of 0·1 *M* sodium phosphate – 0·25 *M* sucrose buffer at *p*H 7·3, 0·4 ml. of 0·1 *M* glucose containing 6·25 mgm. NaCl per ml. and 0·1 ml. of DL-alanine-1-^{14}C solution containing 0·5 mgm. = 0·008 mc. per ml. The latter solution supplies 0·025 mgm. of labelled L-alanine. Samples were incubated for 45 min., at which time 0·2 mgm. of unlabelled D- or L-alanine was added. Samples were then withdrawn at 15-min. intervals, and the activity of the nuclear proteins determined in the usual way

A number of experiments which relate deoxyribonucleic acid to the process of protein synthesis in the nucleus will now be described. Several of these experiments deal with the effect of deoxyribonuclease on the uptake of labelled amino-acids. When isolated nuclei are treated with crystalline pancreatic deoxyribonuclease before adding ^{14}C-amino-acids, the incorporation of the latter is markedly impaired. The results in Table 2 show that the degree of impairment becomes more serious as more and more of the deoxyribonucleic acid is degraded. Removal of practically all the deoxyribonucleic acid leaves only 15 per cent of the activity observed in appropriate 'controls'. These findings suggest a direct role of deoxyribonucleic acid in nuclear protein synthesis.

Additional support for this conclusion arises from experiments which test whether the synthetic activity of nuclei treated with deoxyribonuclease can be restored by the addition of supplementary calf thymus deoxyribonucleic acid. In these experiments the nuclei were treated with deoxyribonuclease under the conditions summarized in Table 3. The activity of the enzyme was then blocked by the addition of citrate, and a deoxyribonucleic acid supplement was added. The isotopic amino-acid was then supplied, and incorporation allowed to proceed for 60 min. The specific activity of the protein in nuclei which had received a deoxyribonucleic acid supplement was then compared with the protein activities in 'control' nuclei which had been treated with the enzyme in the same way but which had not received any additional deoxyribonucleic acid. The results of such experiments all agree in demonstrating that added calf thymus deoxyribonucleic acid does in fact restore a part of the original activity of the nucleus.

It is a matter of considerable interest to determine whether nucleic acids other than calf thymus deoxyribonucleic acid can restore the synthetic capacity of deoxyribonuclease-treated thymus nuclei. Several such experiments are now in progress, and it has been very tentatively concluded that the thymus nucleus can distinguish between thymus deoxyribonucleic acid and that prepared from wheat germ.

Since deoxyribonucleic acid is required for nuclear protein synthesis, the question naturally arises as to how the deoxyribonucleic acid participates in amino-acid incorporation. Several experiments were performed to test whether the action of deoxyribonucleic acid requires the active participation of its free phosphate groups. The results of these experiments are summarized in Table 4. The procedure used was to add varying amounts of basic compounds which are known to combine with the free phosphoric acid

Table 2. Effect of Deoxyribonuclease and Ribonuclease on ^{14}C-Amino-acid Incorporation by Thymus Nuclei

Conditions of experiment*	Isotope administered as	Nucleic acid removed (per cent)	Specific activity of protein (c.p.m./mgm.)	Decrease in ^{14}C incorporation (per cent)
A. Nuclei preincubated with 0·13 mgm. DNase for 15 min. before adding isotopic amino-acid	glycine-1-^{14}C	47	144	30
'Control' nuclei (preincubated without DNase)	,,	—	205	—
B. Nuclei preincubated with 1·0 mgm. DNase for 15 min.	glycine-1-^{14}C	81	33	61
'Control' nuclei	,,	—	84	—
C. Nuclei preincubated with 0·5 mgm. DNase for 30 min.	DL-alanine-1-^{14}C	91	62	66
'Control' nuclei	,,	—	183	—
D. Nuclei preincubated with 0·5 mgm. DNase for 30 min.	DL-alanine-1-^{14}C	>95	2·3	85
'Control' nuclei	,,	—	15·3	—
E. Nuclei preincubated with 0·1 mgm. DNase for 30 min.	DL-alanine-1-^{14}C	—	54	41
'Control' nuclei	,,	—	92	—
F. Nuclei preincubated with 0·3 mgm. DNase for 15 min.	DL-alanine-1-^{14}C	—	51	54
'Control' nuclei	,,	—	109	—
G. Nuclei preincubated with 0·125 mgm. RNase for 15 min.	glycine-1-^{14}C	34	199	3
'Control' nuclei	,,	—	205	—

* In all experiments the incubation flasks contained 1·0 ml. of nuclear suspension, 0·5 ml. of 0·1 *M* sodium phosphate – 0·25 *M* sucrose buffer (*p*H 7·3) and 0·4 ml. of 0·1 *M* glucose containing 6·25 mgm. NaCl and 4·19 mgm. $MgCl_2.4H_2O$ per ml. One-tenth ml. of nuclease solution containing the indicated amount of enzyme was then added and incubation allowed to proceed for the indicated time. The isotopic amino-acid (0·05 mgm.) was then added and incubation continued for 60 min. 'Control' experiments were performed in the same way without adding DNase.

Table 3. Effect of Supplementary Calf Thymus DNA on ^{14}C-Alanine Uptake by DNase-treated Nuclei

	Conditions of experiment*	Calf thymus DNA added (mgm.)	Specific activity of nuclear proteins (c.p.m./mgm.)	Increase in uptake due to added DNA (per cent)
A.	'Control' nuclei (preincubated without DNase)	0	183	—
	" " " " "	2	181	0
	" " " " "	4	183	0
	Nuclei treated with 0·5 mgm. DNase for 30 min. before adding ^{14}C-alanine + citrate	0	62	—
	" " " " " " " "	2	71	15
	" " " " " " " "	4	81	31
B.	'Control' nuclei (preincubated without DNase)	0	92	—
	Nuclei treated with 0·1 mgm. DNase for 30 min. before adding ^{14}C-alanine + citrate	0	54	—
	" " " " " " " "	10	80	48
C.	'Control' nuclei (preincubated without DNase)	0	166	—
	Nuclei treated with 0·1 mgm. DNase for 40 min. before adding ^{14}C-alanine + citrate	0	74	—
	" " " " " " " "	10	101	36
D.	'Control' nuclei (preincubated without DNase)	0	15	—
	" " " "	10	13	—
	Nuclei treated with 0·5 mgm. DNase for 30 min. before adding ^{14}C-alanine + citrate	0	2·3	—
	" " " " " " "	10	4·8	108

* In these experiments the incubation flasks contained 1·0 ml. of nuclear suspension, 0·5 ml. of 0·1 *M* sodium phosphate – 0·25 *M* sucrose buffer (*p*H 7·3) and 0·4 ml. of 0·1 *M* glucose containing 8·16 mgm. NaCl and 4·19 mgm. $MgCl_2.4H_2O$ per ml. 0·1 ml. of DNase solution containing the indicated amount of enzyme was then added and incubation allowed to proceed for the indicated time. Then 1 ml. of DNA solution containing the indicated amounts of calf thymus DNA and 20 μM of sodium citrate was added, followed by 0·1 ml. (= 0·05 mgm.) of DL-alanine-1-^{14}C solution. Incubation was continued for 60 min. 'Control' experiments were performed in the same way without adding DNase.

groups of deoxyribonucleic acid. The compounds selected included the basic dye, methyl green, and two basic proteins, protamine, and a lysine-rich histone prepared from calf thymus[10]. It is clear that the addition of small amounts of methyl green, protamine or histone to the nuclei before adding the isotopic amino-acid has little effect upon the uptake. Larger amounts lead to a partial impairment of carbon-14 incorporation; but the impairment may reflect osmotic or other damage to the system, since large amounts of added serum albumin also decrease the uptake.

A few other observations are included in Table 4. The first deals with the effect of chloramphenicol, which has been shown to block protein synthesis in bacterial systems[11]. This compound had little effect on amino-acid incorporation by isolated thymus nuclei. A second observation is concerned with the effect of added cortisone, which has been shown to cause involution of the thymus *in vivo*[12]. The addition of this steroid diminishes amino-acid uptake by the isolated nuclei.

Since deoxyribonucleic acid is required for protein synthesis in the nucleus, and ribonucleic acid is needed

Table 4. Effects of Basic Substances, Cortisone and Chloramphenicol on ^{14}C-Amino-acid Incorporation by Isolated Thymus Nuclei

	Conditions of experiment	Isotope administered as	Specific activity of protein (c.p.m./mgm.)	Change in ^{14}C uptake due to added compound (per cent)
A.	'Control' nuclei	DL-alanine-1-^{14}C	154	—
	Nuclei + 0·125 mgm. methyl green	"	167	+ 8
	" + 0·25 " " "	"	162	+ 5
	" + 0·50 " " "	"	116	−25
B.	'Control' nuclei	glycine-1-^{14}C	205	—
	Nuclei + 0·25 mgm. methyl green	"	251	+22
C.	'Control' nuclei	glycine-1-^{14}C	89	—
	Nuclei + 0·50 mgm. methyl green	"	64	−28
D.	'Control' nuclei	DL-alanine-1-^{14}C	154	—
	Nuclei + 0·375 mgm. protamine	"	168	+ 9
	" + 0·75 " "	"	159	+ 3
	" + 1·50 " "	"	164	+ 6
E.	'Control' nuclei	glycine-1-^{14}C	79	—
	Nuclei + 1·5 mgm. protamine	"	70	−11
	" + 3·0 " "	"	31	−61
	" + 1·5 mgm. histone (lysine-rich)	"	76	− 4
	" + 3·0 " " "	"	62	−21
	" + 2·63 " arginine	"	63	−20
	" + 3·0 " bovine serum albumin	"	66	−16
F.	'Control' nuclei	glycine-1-^{14}C	75	—
	Nuclei + 0·5 mgm. protamine	"	73	− 3
	" + 1·0 " "	"	70	− 7
	" + 2·0 " "	"	45	−40
	" + 1·75 " arginine	"	62	−17
	" + 2·0 " bovine serum albumin	"	62	−17
G.	'Control' nuclei	glycine-1-^{14}C	21	—
	Nuclei + 0·1 mgm. chloramphenicol	"	19	−10
H.	'Control' nuclei	DL-alanine-1-^{14}C	217	—
	Nuclei + 0·063 mgm. cortisone	"	138	−36
	" + 0·125 " "	"	122	−54

for protein synthesis in the cytoplasm, the question naturally arises as to the role of ribonucleic acid within the nucleus. Attempts to relate the ribonucleic acid of the thymus nucleus to nuclear protein synthesis have not so far been successful. Treatment of the isolated nuclei with ribonuclease before adding ^{14}C-amino-acids has no effect on the uptake of the latter. However, this result does not permit the conclusion that ribonucleic acid has no part in nuclear protein synthesis, because in the experiments mentioned only 30 per cent of the nuclear ribonucleic acid was released by the action of the enzyme.

One difficulty which should be mentioned in this connexion is the problem of nuclear variability. Nuclei prepared from different thymus glands at different times of the year may vary considerably in their properties. One way in which they vary is in their susceptibility to ribonuclease. Several preparations (made during the winter months) released all their ribonucleic acid when treated with the enzyme, whereas nuclei prepared more recently were relatively resistant. The nuclear variability is evident in other properties as well: in osmotic properties, as evidenced by the tendency to form gels, and in the ability to retain the complement of acid-soluble nucleotides.

Which nuclear proteins become labelled? The answer to this question depends at the outset upon the existence of a dependable and meaningful scheme of fractionating the proteins of the nucleus. This is a field of endeavour which is now in its earliest stages of development, though it promises to become a major topic of biochemical investigation.

We have developed a provisional fractionation procedure which is applicable to the proteins of thymus 'sucrose' nuclei, and which permits the separation of several classes of proteins of different properties, and considerable biological interest. In its essentials the procedure is as follows. (See flow-sheet in Fig. 6.) The nuclei are first extracted with *p*H 7·1 potassium phosphate buffer. The extractable protein (Fraction 1) is about 10 per cent of the whole nuclear mass and contains about one-third of the ribonucleic acid of the nucleus. The nuclei are then suspended in 1 *M* sodium chloride, in which

FRACTIONATION OF THE PROTEINS OF ISOLATED CALF THYMUS NUCLEI

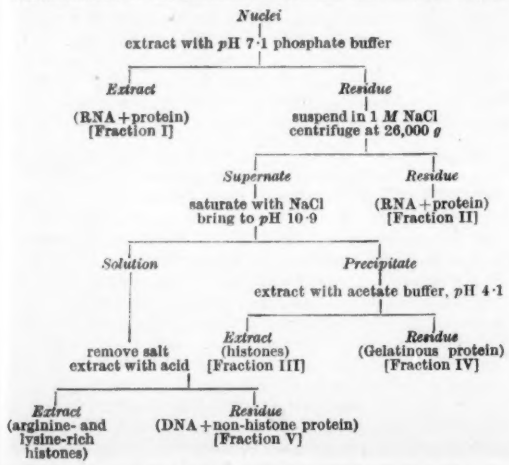

Fig. 6. Flow-sheet for the fractionation of the proteins of calf thymus 'sucrose' nuclei

Table 5. ALANINE-1-^{14}C INCORPORATION INTO DIFFERENT PROTEIN FRACTIONS OF THYMUS NUCLEI

Protein fraction	Specific activity (c.p.m./mgm.)
Total nuclear protein	107
Fraction I (protein soluble in *p*H 7·1 buffer)	179
Fraction II (residue after extraction in *M* sodium chloride)	117
Fraction III (histones)	36
Fraction IV (gelatinous protein)	157
Fraction V (non-histone protein associated with DNA)	243

they swell and largely dissolve. The opalescent solution is centrifuged at 26,000 *g* to sediment the undissolved protein. This residue (Fraction II) also comprises about 10 per cent of the nuclear mass and contains one-third of the total ribonucleic acid. The supernatant after high-speed centrifugation contains the bulk of the protein of the nucleus and all the deoxyribonucleic acid. This solution is saturated with sodium chloride and brought to *p*H 10·9. The resulting precipitate is treated with acetate buffer at *p*H 4·1 to extract a histone fraction (Fraction III), leaving a gelatinous protein residue (Fraction IV). This gelatinous protein constitutes about 8 per cent of the nuclear mass. Its amino-acid composition and other properties show that it is not a histone. After removing the salt from the saturated sodium chloride supernatant, arginine-rich and lysine-rich histones[10] are removed in dilute acid. The residue contains all the deoxyribonucleic acid of the nucleus and a firmly attached protein fraction (Fraction V) the amino-acid composition of which shows that it is not a histone.

When nuclei are incubated in the presence of ^{14}C-alanine and afterwards fractionated according to the above scheme, wide variations in specific activity are observed in the different protein fractions. (It should be mentioned that the total alanine contents of the protein fractions are very similar, so that differences in carbon-14 concentration reflect differences in the metabolic activity of the various proteins.) The results of such an experiment are presented in Table 5. Several of the findings are of particular interest. First is the observation that the protein (Fraction V) which is most closely associated with the deoxyribonucleic acid is by far the most 'active' protein of the nucleus. This result lends further support to the conclusion drawn from deoxyribonuclease experiments that the deoxyribonucleic acid plays a direct part in protein synthesis. A second point of interest is the fact that the level of isotope incorporation into the histone protein (Fraction III) is relatively low. This result agrees with earlier *in vivo* experiments[12] in which it was noted that the incorporation of glycine-^{15}N into the histones of mouse liver, pancreas and kidney is much lower than the uptake into the residual proteins of the chromosome. Finally, it may be noted that the ribonucleoprotein complex which is extracted in *p*H 7·1 phosphate buffer has a high level of activity. It is tempting to speculate that this high activity in a protein fraction of the nucleus which is diffusible indicates a role of this protein in the mechanism by which the nucleus interacts with the cytoplasm.

Since a protein component of ribonucleoprotein can be synthesized in the isolated nucleus the question arises as to whether the isolated nucleus can also synthesize ribonucleic acid, and whether the ribonucleic acids in different fractions are synthesized at different rates. (It has already been reported that

pig kidney nuclei isolated in sucrose can incorporate ^{32}P-orthophosphate into their ribonucleic acid[14].) The ability of thymus and lymphoma nuclei to utilize glycine-^{14}C for ribonucleic acid synthesis, and the role of deoxyribonucleic acid in this process are currently being investigated. Preliminary experiments show that ^{14}C-glycine is actively incorporated into the purines of the nucleic acids of isolated thymus nuclei.

Energy-yielding Mechanisms in Isolated Nuclei

As considerable energy should be required for the active synthesis of protein in the nucleus, one would expect nuclei to contain an energy generating system, possibly involving energy-rich compounds such as adenosine triphosphate and related nucleotides. It has already been pointed out that the incorporation of ^{14}C-amino-acids is an aerobic phenomenon, inhibited by both cyanide and dinitrophenol.

To determine the overall concentration and distribution of the nucleotides in thymus nuclei, analyses were first performed on the 'standard', that is, nuclei prepared in non-aqueous media by the Behrens' procedure. The cold acid extract of Behrens' type nuclei was fractionated by ion-exchange chromatography[15], and it was found that these nuclei contain mononucleotides of adenine, cytosine, uracil and guanine, together with several uridine diphosphate derivatives, the adenine nucleotides predominating in amount. The nucleotide analyses of the Behrens' type nuclei are in close agreement both quantitatively and qualitatively with similar analyses performed on whole tissue, indicating that in this respect at least no gross differences exist between nucleus and cytoplasm (Table 6).

When similar analyses are performed on thymus nuclei isolated in sucrose solutions it is found that they retain 60–80 per cent of their original acid-soluble nucleotide content. These nucleotides are well retained after repeated washings with cold sucrose – calcium chloride solution. From the analyses of the nucleotides found in thymus nuclei, a surprising observation came to light. During the preparation of 'sucrose' nuclei in the cold, the nucleotide monophosphates characteristic of the excised tissue are largely converted back to the energy-rich triphosphate forms which predominate in the living tissue. This synthesis of high-energy phosphate bonds is presumably the result of extensive aeration of the nuclear suspension during the isolation procedure.

To test whether the synthesis of compounds like adenosine triphosphate is an intranuclear phenomenon, or whether it requires the participation of the cytoplasm, the following experiment was performed. Nuclei were isolated as quickly as possible (25 min. at 2° C.). At this time, most nucleotides are still present as monophosphates (see Table 6). The isolated nuclei were then stirred gently in the cold for an additional 60 min. At the end of the experiment almost all the nucleotides had been converted to the triphosphate form. Since the conversion takes place in isolated nuclei, and occurs regardless of the volume in which the nuclei are suspended, it appears that this is an intranuclear synthesis of high-energy phosphate. Only intranuclear adenosine monophosphate seems to be involved because added adenosine monophosphate is not acted upon. The results in Table 6 also make it clear that the process is completely inhibited in the presence of 10^{-3} *M* sodium cyanide or 2×10^{-4} *M* dinitrophenol. However, it should be noted that certain reagents which block oxidative phosphorylation in mitochondria, namely, janus green *B* (2×10^{-4} *M*), methylene blue ($2{\cdot}5 \times 10^{-4}$ *M*), and dicumarol (10^{-4} *M*), have no effect on the synthesis of adenosine triphosphate by isolated thymus nuclei. Synthesis of adenosine triphosphate does not occur in the absence of oxygen. Taken together, the results indicate that nuclei are equipped with energy-generating systems which presumably operate via some type of oxidative mechanism. The relationship of this to the mitochondrial system is being considered.

Table 6. Adenine Nucleotide Contents of Isolated Calf Thymus Nuclei

	Material analysed	Per cent of total $E_{.m}$ present as AMP	ADP	ATP
A.	Nuclei prepared by Behrens' method*	19·0	4·5	3·3
	Whole thymus tissue*	20·6	5·7	3·4
B.	Nuclei prepared in 0·25 *M* sucrose (isolation requiring 45 min.)	9·3	7·7	14·0
C.	'Sucrose' nuclei after rapid isolation (25 min. at 2° C.)	19·0	10·5	7·6
	Above nuclei stirred gently at 2° for 60 min. after isolation	3·5	5·7	24·5
D.	'Sucrose' nuclei prepared in 10^{-3} *M* sodium cyanide	21·5	4·9	3·5
	'Control' nuclei prepared without cyanide	8·5	6·3	19·1
E.	'Sucrose' nuclei prepared in 2×10^{-4} *M* dinitrophenol	20·1	8·0	< 1·1
	'Control' nuclei prepared without DNP	8·6	5·6	15·0

* The predominance of nucleotide monophosphate in thymus tissue and in thymus nuclei prepared by the Behrens' procedure should not be taken to indicate the actual conditions *in vivo*. It is probable that the predominance of these low-energy forms is simply a reflexion of anoxia resulting from the death of the animal. Evidence to support this conclusion stems from the nucleotide analyses of rat thymus which was frozen in liquid nitrogen immediately upon removal. In this instance the nucleotide triphosphates predominate over di- and mono-phosphates.

Amino-Acid Incorporation by Lymphoma Cell Nuclei

The procedures described for the study of protein synthesis in isolated thymus nuclei have recently been extended to include another test system. Nuclei have been prepared from the cells of a transplantable mouse lymphoma [*AKR* lymphoma *L* 1]. (This material was very generously given to us by Dr. John G. Kidd, of Cornell University Medical College.) The time-course of incorporation of ^{14}C-alanine by the lymphoma nuclei is included in Fig. 1.

In summary, nuclei prepared from calf thymus in a sucrose medium actively incorporate ^{14}C-labelled amino-acids. It has been shown that this is a synthetic reaction which requires the participation of the deoxyribonucleic acid of the nucleus. Therefore a biochemical function of deoxyribonucleic acid is the synthesis of protein in the chromosome. The free phosphate groups of the deoxyribonucleic acid do not seem to be involved in the process. When the proteins of the nucleus are fractionated and then classified according to their specific activities, one finds the histones to be relatively inert. The protein fraction most closely associated with the deoxyribonucleic acid has the highest activity. A diffusible ribonucleoprotein complex is also active. Together with earlier observations on the incorporation of amino-acids by cytoplasmic particulates, these results show

that protein synthesis can occur in both nucleus and cytoplasm. It also follows that the nucleus can meet the energy requirements of protein synthesis. In this connexion it has been found that the isolated thymus nucleus is endowed with systems which generate high-energy phosphate bonds. [July 26

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THE VENEZUELAN INSTITUTE FOR NEUROLOGY AND BRAIN RESEARCH

By Dr. GEOFFREY H. BOURNE
London Hospital Medical College

SITUATED in the beautiful mountains surrounding Caracas, the capital of Venezuela, and only 12 km. away from the centre of the city, is an Institute which represents the first stage of a great scheme for neurological research.

On January 1, 1955, the 5,000-ft. mountain on which this Institute has been placed was virgin territory; since then, the prodigious efforts of Dr. Humberto Fernandez-Moran have resulted in the flattening of the top of the mountain, the building of an 800-kW. electric power station with specially controlled voltage, a water-supply system which includes an artificial lake of some 6 million litres capacity, and what is described as the 'Pilot' unit of the Institute, together with residences for the staff. On December 2 this first section of the new Institute was being opened by the President of Venezuela, General Marcos Perez Jimenez, less than a year from the arrival of the first bulldozer on the mountain top.

This first stage of the plan has already cost three million dollars, but much more will be spent before the entire project is completed. This first part contains laboratories for research into nerve ultrastructure, neurophysiology, biophysics, biochemistry and biomathematics. In addition, there are a large library, several workshops and ten residential units for scientific personnel and assistants. A guest house for visitors from abroad is also incorporated into the design. Further development of the Institute envisages a large building for the Neurotropic Virus Research Unit, a large radiation laboratory which will be equipped with a nuclear reactor for medical research, and further buildings for biochemistry, biomathematics and experimental neuropharmacology. It is proposed ultimately to have a hospital of two

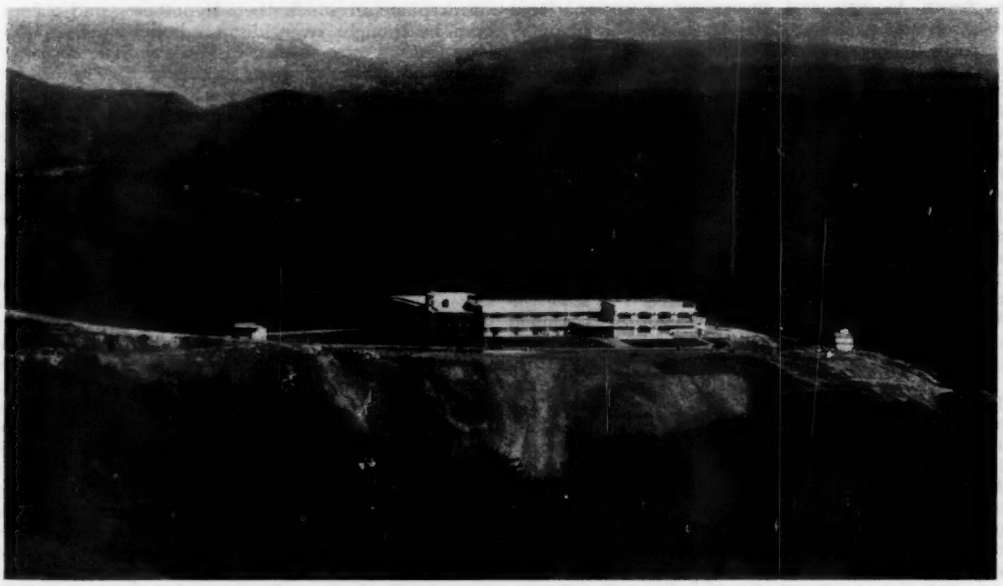

Fig. 1. The Venezuelan Institute for Neurology and Brain Research

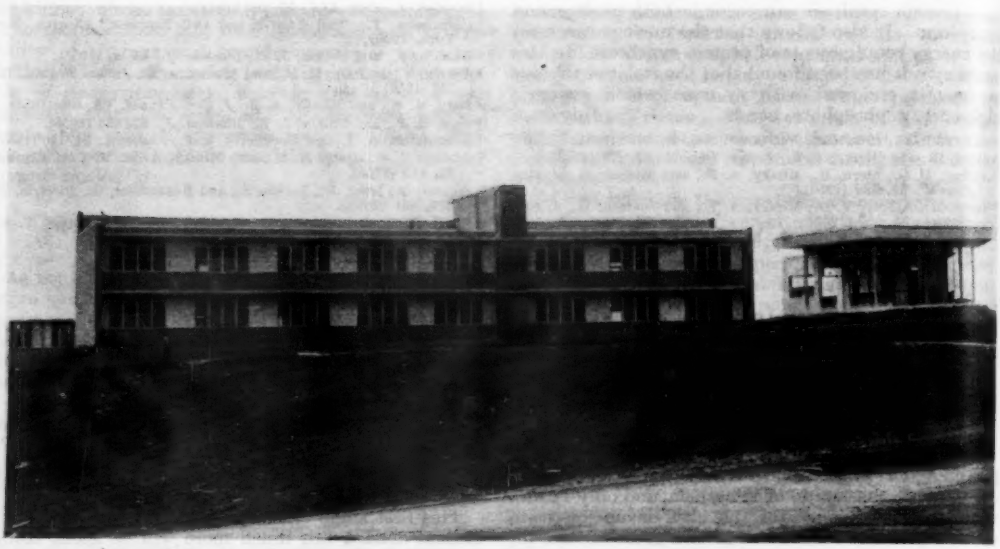

Fig. 2. First building of IVNIC (Venezuelan Institute for Neurology and Brain Research) containing: (1) Department of Nerve Ultrastructure (equipped with three electron microscopes); (2) Department of Neurophysiology; (3) Neurotropic Virus Research Unit; (4) Department for Nuclear Magnetic Resonance Studies; (5) Department of Biophysics; (6) Department of Biomathematics

hundred beds similar to the Research Hospital at the National Institutes of Health in Bethesda, U.S.A. In fact, the Institute was founded last year (April 29, 1954) by decree of the President of Venezuela as an autonomous government agency similar in status to the National Institutes of Health, and in many respects to the National Institute for Medical Research in Great Britain.

The object of the Institute goes beyond being simply a national institution; it is hoped that it will become a regional research centre in neurology in South America and eventually an international research and training centre in this field. In many respects it is strategically situated for this purpose, being only seven to eight hours away by aeroplane from New York and fifteen to eighteen hours away from Britain and Western Europe. Close contact with the United States has already been established by the signature on June 14 last of an agreement between the two countries under the terms of which the United States will furnish Venezuela and the Institute with certain radioactive materials and will supply information on the design, construction, operation and use of nuclear reactors for research. The U.S. Atomic Energy Commission, under the terms of this agreement, has also agreed to lease the Venezuelan Government up to 6 kgm. of contained uranium-235 in uranium enriched up to a maximum of 20 per cent uranium-235.

The proposed international character of the Institute has been emphasized by the first staff appointments: Dr. Gunnar Svaetichin, of the Karolinska Institute in Sweden, has already arrived in Caracas to take charge of the Department of Neurophysiology, which has been organized and equipped especially to work with the refined microelectrode techniques with which Dr. Svaetichin carried out pioneer work in Sweden. In addition to this, a department devoted to the application of nuclear magnetic resonance technique to the study of paracrystalline biological components like the myelin sheath of nerves is being organized with the collaboration of Dr. P. Dennis, of the University of Geneva. Full staffing of the various departments of the Institute will be a slow process, and will obviously have to be carried out with care. Those who do go to the new Institute may well find that the perpetual springlike climate of the mountain-top, on which the Institute lies, the feeling of peaceful detachment from the world engendered by a mountainous habitat, the congenial colleagues and lack of financial worries provided by a generous salary, will provide a unique atmosphere in which their hypotheses and theories can be thought over, discussed and finally put to the test.

Providing an adequate library for the staff is a problem which the Institute will need to solve. Back numbers of many journals would be difficult to obtain; but it is proposed for this purpose to build up a collection of microfilms of back numbers. The policy of the library will also be to invest in current journals rather than books, since books are static, rapidly becoming out of date, whereas journals are the life-stream of advancing knowledge. The equipping of the Institute will also be important; but donors have been generous for this purpose, and the researches on fine structure of nerves will have the benefit, for example, of three electron microscopes.

A description of this remarkable new Institute would be incomplete without some reference to its initiating genius and first director, Dr. Fernandez-Moran. Dr. Fernandez-Moran graduated in medicine in Munich and later re-qualified at the Central University, Caracas. Afterwards he took a Ph.D. in physics in the University of Stockholm. He was a fellow in neurology at George Washington University, Washington, D.C., assistant in neurosurgery at the Serafimerlas Clinic, Stockholm, and Research Fellow at the Institute for Cell Research, Karolinska Institute, Stockholm, and assistant professor of cell research in the same Institute. As well as being the first director of the new Institute, he holds the chair

of biophysics at the Central University, Caracas. His electron microscope studies have established his reputation as an authority on the sub-microscopic organization of vertebrate nerve fibres. Much of this work was due to his development of a microtome for cutting sections of nervous and other tissues (including undecalcified bones and teeth) at thicknesses of 100 A. or less, which are necessary for good electron microscope investigations. For this machine he also designed a diamond knife which could be made from industrial diamonds. In the Institute, Dr. Fernandez-Moran will take charge of the work on fine structure of nervous tissue, and he has established a special department to continue the development work on the diamond knife and on microtomes to use with them.

During my visit to the Institute last summer, it was a revelation to see the energy and speed with which Dr. Moran tackled and coped with the innumerable details of equipping and starting the Institute in time for the opening.

Venezuela is in an extremely fortunate financial position due to the exploitation of her oil and other natural resources, and she is extremely fortunate that she has at such a moment someone like Dr. Fernandez-Moran, who was able to conceive and to execute such a remarkable scheme, and an enlightened Minister of Health and President, who were far-sighted enough to see that money invested in this way would benefit not only Venezuela itself but ultimately the rest of the world as well. The Venezuelan Institute for Neurology and Brain Research is a practical step forward in international scientific research.

OBITUARIES

Dr. J. W. Nicholson, F.R.S.

Dr. J. W. Nicholson, an outstanding mathematician and man of science, died on October 10, aged seventy-three. He was the eldest son of the late J. W. Nicholson, of Redcar, Yorkshire, and began the serious study of mathematics and physical science at the University of Manchester. Later, he entered Trinity College, Cambridge, where his brilliant career began. He was 12th Wrangler in 1904 and Isaac Newton Student in 1906. In 1907 he was Smith's Prizeman. He was Adam's Prizeman twice—in 1913 and 1917. He lectured at the Cavendish Laboratory and later at The Queen's University, Belfast.

In 1912 he was appointed professor of mathematics in the University of London, King's College, and occupied this chair until 1921, when he became tutor at Balliol College, Oxford, where he was director of studies in mathematics and physics until serious illness forced him to retire in 1930.

Nicholson was one of the most brilliant mathematicians and astrophysicists of his time. In 1917 he was elected into the Royal Society. He was also a Fellow of the Royal Astronomical and the London Mathematical Societies, past president of the Röntgen Society, a former vice-president of the Physical Society and a member of the Société de Physique. He was an excellent lecturer and teacher, and probably no teacher in King's College in his time was so much appreciated and admired by his students and colleagues as he was.

Nicholson's scientific achievements are much more important than is generally realized in these days: the elucidation of the electronic structure of atoms owes as much to Nicholson as to any other one man. Before his appointment to King's College, he became interested in certain nebular spectra (the nebula in Orion among others) and the spectrum of the solar corona; and the first of a most remarkable sequence of papers on this subject appeared in the *Monthly Notices of the Royal Astronomical Society* (**72**, 49; 1912), entitled "The Spectrum of Nebulium". In this he attributed certain nebular spectral lines to a primitive type of atom which he named nebulium. This he imagined to consist of a small central massive positively charged nucleus with four electrons travelling around it on the same circular orbit. He was thus enabled to account for certain spectral lines which, it seemed, could not be ascribed to hydrogen or helium. His work was inspired by Clerk Maxwell's famous paper on Saturn's rings. He was, in fact, able to identify a large number of nebular spectral lines with those which his nebulium would be expected to emit. Indeed, he *predicted* a nebular spectral line the wave-length of which he calculated to be 4352·9 A. before it was observed. It was later observed and measured by Prof. F. J. M. Stratton, who estimated it to be 4353·3 A.

Perhaps the most remarkable feature of Nicholson's work was the discovery that angular momentum is an integral multiple of $h/2\pi$. There is no doubt that this work inspired his pupil, Niels Bohr, to explore the consequences of assuming the hydrogen atom to have a single electron travelling in a circle around a massive nucleus, and it was certainly part of the inspiration of the $\int pdq = nh$ condition enunciated by one of his colleagues at King's College and which was later adopted by Bohr.

One must not omit to mention the important work in which Nicholson collaborated with the distinguished spectroscopist, Sir Thomas Merton, and which was published in the *Philosophical Transactions of the Royal Society* (A, **216**, 459; 1916; A, **217**, 237; 1917; and A, **220**, 137; 1919). It dealt with the distribution of intensity in broadened spectral lines and with intensity relations in the spectra of hydrogen and helium.

Nicholson was a friendly and generous man who took great pleasure in being helpful to both students and colleagues. He was a man of high character and in that respect reminded me of our colleague, H. Stanley Allen, and of the great Max Planck. All of us who knew him were deeply distressed by his tragic illness. As a mathematician he impressed me as very sound, and I learned more from him than from any other man, with the exception, perhaps, of the German mathematician, Carl Neumann. I shall always treasure his memory.

Nicholson married Dr. Dorothy Wrinch, Fellow of Girton College, Cambridge, in 1922, and had one daughter; the marriage was dissolved in 1938.

Wm. Wilson

Mr. Alexander Keiller

Alexander Keiller, who died at the end of September at the age of sixty-five, was one of the long line of distinguished amateur British archæologists which goes back to Aubrey and Stukeley. Of these perhaps the greatest was Pitt-Rivers, on whom Keiller, like him with leisure and abundant means at his disposal, admittedly modelled his own work.

While still in his early thirties, Keiller carried out a systematic survey of stone circles and allied monuments in north-east Scotland, making accurate plans and detailed descriptions of each site. A summary account of this work was presented as a paper to the British Association at its Aberdeen meeting in 1934.

In 1925, however, he embarked on the programme of excavation and field-work with which his name will be inseparably associated. He acquired and began excavations on Windmill Hill, near Avebury in north Wiltshire, and continued these annually until 1929, revealing in detail the first extensive Neolithic settlement to be explored in Britain. The material was housed in a museum in his London house, where it was at the disposal of students, and as a result the earliest Neolithic culture of southern Britain, taking its name from the type-site, was defined and clarified.

From Windmill Hill to the Avebury monuments themselves was an inevitable step, and in 1933 Keiller began a systematic excavation programme with the examination of the northern part of the West Kennet Avenue, continuing until 1938 within the Great Circle itself. He moved his residence to Avebury Manor, and re-housed his museum within its grounds, making it and the excavated portions of the monument available to the public. He adopted a policy of imaginative but judicious conservation and restoration of the Avebury monuments, and systematically purchased land to preserve these and their surroundings; as a result it was eventually possible for the whole area to be acquired by the National Trust and the Ancient Monuments Department of the Ministry of Works.

Keiller's outstanding contribution to contemporary British archæology was his insistence on high standards of discipline and accuracy in excavation and field-work, and his realization from the first of the value of scientific techniques as applied to archæological material. He early appreciated the potentialities of air photography, collaborating with Crawford in the "Wessex from the Air" survey, and even discussing with Eckener the possibility of using the *Graf Zeppelin* for a similar but even more ambitious scheme. At Windmill Hill he insisted on a full study of the faunal and floral remains in their archæological context. But above all it is to Keiller that we owe the inception of the systematic study of British prehistoric stone artefacts by petrographical means, which, following his lead, is now yielding information of the highest importance on manufacture and trade in the early second millennium B.C. Alec Keiller was an enthusiast in the best sense; full of ideas, stimulating and highly individual. Archæology, like other academic disciplines, can only benefit from the impact of such men.

STUART PIGGOTT

Dr. E. J. Delporte

DR. E. J. DELPORTE, who died at Uccle on October 19, was honorary director of the Royal Observatory at Brussels, where he had continued to work since his retirement from the directorship in 1947.

Eugène Joseph Delporte was born in Brabant in 1882, and after studying mathematics and physics at the University of Brussels, obtained the degree of D.Sc. with distinction in 1903, entering the Observatory in the same year. In 1923 he took charge of the equatorial instruments and began that programme of minor planet observations which was to become his main interest. It was during this period that Delporte proposed that new boundaries should be adopted for the constellations, defined in terms of hour circles and parallels of declination. The proposal was accepted by the International Astronomical Union in 1928, and the work was completed in 1930. Originally intended to apply only to constellations north of 12·5° south declination, the scheme finally covered the sky from pole to pole, the northern boundaries merging well with those which Gould had previously defined for the southern hemisphere.

In 1936 Delporte was appointed director of the Royal Observatory at Brussels, and pursued a vigorous policy of systematic observations of the minor planets. It is impossible to estimate the number of new discoveries that were made during these years, but some dozens of these minor planets have received permanent numbers. Planet 1247 carries Delporte's name, as does the comet 1941 VII; but he will probably be best remembered as the discoverer of the two remarkable asteroids Amor (1221) and Adonis, the orbits of which are still exceptional in a list of more than 1,600.

Delporte worked enthusiastically for the International Astronomical Union from its first meeting in Brussels in 1919, and played a prominent part in Commission 20 (minor planets, comets and satellites), of which he was president during 1938–48. He received many decorations from the hands of his countrymen, and was an active member of many scientific societies with astronomical interests. He was a corresponding member of the Bureau des Longitudes and of the Paris Academy of Sciences, and in 1946 was elected an associate of the Royal Astronomical Society.

Conscious always of the needs of his Observatory, Delporte was mainly responsible for the improvements which were effected in 1930, on the occasion of the centenary of the independence of Belgium, when a 1-metre reflector, a double astrograph and a new meridian circle were added to the equipment. Always ready to praise the scientific success of his colleagues, he gave every encouragement to the younger members of his staff, who will long remember him as a remarkable observer and a wise and friendly counsellor.

J. G. PORTER

Prof. Robert P. Bigelow

THE death occurred on September 6 of Prof. Robert P. Bigelow, who retired in 1933 after forty years service in the Biology Department of the Massachusetts Institute of Technology; he was ninety-two years old. A native of Baldwinsville, New York, Dr. Bigelow graduated from Harvard University and received the degree of doctor of philosophy at Johns Hopkins University before joining the staff of the Massachusetts Institute in 1893 as an instructor. He was appointed assistant professor of zoology and parasitology in 1912, associate professor in 1915, and professor in 1922. Following his retirement, Dr. Bigelow served as honorary lecturer in the Biology Department; and, in 1944, returned as a special lecturer. He was librarian of the Institute during 1895–1925. Dr. Bigelow was also the former librarian of the Marine Biological Laboratory at Woods Hole, and editor of the *American Naturalist* and the *Technology Quarterly*.

NOBEL PRIZES FOR 1955

THE following awards of Nobel Prizes to scientific men have been announced:

Physics: Profs. P. Kusch and W. E. Lamb

The joint award of the Nobel Prize for Physics for 1955 to Prof. P. Kusch, of Columbia University, and Prof. W. E. Lamb, of Stanford University, recognizes a common feature in their contributions to physics. Both have applied the methods of high-precision radio-frequency spectroscopy to atomic phenomena, in which the effect on the electrons of their own radiation field, though very small, is significant.

Prof. Kusch has applied the methods of radio-frequency resonance in atomic and molecular beams to determine, with the highest achievable precision, the magnetic moment of atomic nuclei, and of the electron. In this way he has shown that the spin-moment of the electron is not precisely one Bohr magneton, but $(1{\cdot}001146 \pm 0{\cdot}000012)$ Bohr magnetons. Modern theories, developed at about the same time, and greatly stimulated by the experimental work of Kusch and of Lamb, predict the value $1 + e^2/hc - 11{\cdot}89\ e^4/h^2c^2 + \ldots = 1{\cdot}00114536$. Kusch's measurements of nuclear magnetic moments, from nuclear resonance, and from atomic hyperfine structure, show small but significant discrepancies which throw light on the structure of atomic nuclei.

Prof. Lamb, while primarily a theoretical physicist, has turned to experiment to test the prediction of modern theories of quantum electrodynamics, that the fine structure of the hydrogen spectrum is modified by the reaction of the electron with its radiation field. According to the simple Dirac wave equation, the energy of the metastable $2s_{\frac{1}{2}}$ level of hydrogen is exactly equal to the energy of the $2p_{\frac{1}{2}}$ level. In an experiment where transitions are induced between these two levels in an atomic beam, Lamb demonstrated that they are separated by $1057{\cdot}77 \pm 0{\cdot}10$ Mc./s. Modern theories predict $1057{\cdot}19 \pm 0{\cdot}16$ Mc./s.

Prof. Lamb has recently accepted appointment to the chair of theoretical physics in Oxford.

Physiology and Medicine: Prof. Hugo Theorell

The Nobel Prize for Physiology and Medicine for 1955 has been awarded to Prof. Hugo Theorell, director of the Biochemical Department of the Medical Nobel Institute of Stockholm, for his work on flavoprotein enzymes and hæmoproteins such as cytochrome *c*, peroxidase, catalase and myoglobin. The study of the "old flavoprotein enzyme", the prosthetic group of which is flavin mononucleotide (FMN), was carried out by him when he was holding a Rockefeller Foundation Fellowship at the Kaiser Wilhelm Institute (now Max Planck Institute) for Cell Physiology (Berlin-Dahlem). It is the electrophoretic separation of this enzyme from a polysaccharide, which accompanied it, that enabled him to purify this enzyme and to determine its main properties. Of special interest, however, is his extensive study of cytochrome *c*, the extractable and soluble component of the cytochrome system which constitutes the intracellular respiratory chain of catalysts linking the dehydrogenase systems with molecular oxygen, thus providing the essential path for hydrogen or electron transfer from the activated molecules of substrates to oxygen. To the study of the physico-chemical properties of cytochrome *c* and other hæmoproteins, the mechanism of the reactions in which they are involved and the nature of the links between their prosthetic groups and protein, Prof. Theorell has applied great technical skill and a variety of modern physico-chemical and biochemical methods.

More recently, Prof. Theorell and his co-workers studied the properties of alcohol dehydrogenase extracted and purified from liver, and they were able for the first time to follow spectrophotometrically the kinetics of reactions between the enzyme and the reduced co-enzyme I (diphosphopyridine nucleotide). From this work emerged a method for the precise estimation of minute amounts of alcohol, which is of great value for medico-legal work. Prof. Theorell has established a very active centre of research which attracted many workers from different countries to his department which, in 1948, was transferred to the new building of the Medical Nobel Institute, where it is provided with all facilities for the study of a great variety of biophysical and biochemical problems.

Chemistry: Prof. Vincent du Vigneaud

This year's Nobel Prize for Chemistry goes to Prof. Vincent du Vigneaud, professor and head of the Department of Biochemistry, Cornell University Medical College, New York. Prof. du Vigneaud had many achievements to his name before his recent outstanding work on the structure and synthesis of oxytocin and vasopressin. His researches on the chemistry of insulin were a valuable early contribution in this difficult subject, and were naturally related to his studies on intermediary metabolism and sulphur-containing substances. A distinguished series of investigations on the mechanism of transmethylation and the biosynthesis of choline were based on his substantial experience of both chemical and biochemical methods and greatly added to our knowledge of the metabolism of methionine and of cystine. During the Second World War, Dr. du Vigneaud turned his attention to the chemistry of penicillin, and his synthesis of this substance was a significant contribution to this subject. More recently his activities, and those of his colleagues, have been largely directed to the isolation, chemistry and synthesis of the posterior pituitary hormones, oxytocin and vasopressin. The demonstration that these substances are closely related octapeptides, each containing a disulphide bond spanning six amino-acid residues (a type of structure also found in insulin by Sanger), and the fact that, as long ago as 1935, du Vigneaud had shown that oxytocin can be reduced and reoxidized without obvious inactivation, paved the way to the synthesis of both of these hormones. Each step in a series of complex investigations has been checked with the utmost care, and the complete synthesis of these two related substances has opened wide the door to vast new developments in the pharmacology of peptide hormones and their chemical relations.

NEWS and VIEWS

Wellcome Laboratories of Tropical Medicine, London : Brigadier J. S. K. Boyd, O.B.E., F.R.S.

Brigadier J. S. K. Boyd has resigned his position as director of the Wellcome Laboratories of Tropical Medicine as from January 1, 1956, on his appointment as a Wellcome trustee in place of Prof. T. R. Elliott, who has retired. Brigadier Boyd joined the Royal Army Medical Corps in 1914 and served in France, Macedonia and India. In 1929 he carried out researches in India on a variety of pathological problems. Returning to England, he was principally responsible for the official adoption of immunization with tetanus toxoid in the Army, a practice which was almost to eliminate casualties from tetanus during the Second World War. During the War he was deputy director of pathology to the Middle East Forces in Egypt, where he was concerned with the organization of blood transfusion services and the many pathological problems arising in a subtropical climate. Among these problems the introduction of sulphaguanidine, in collaboration with Sir Neil Hamilton Fairley, for the treatment of bacillary dysentery was outstanding. He became deputy director of pathology to 21 Army Group in 1944 and served in that capacity in Western Europe until 1945, when he became director of pathology to the War Office. In 1946 he resigned this position to accept appointment as director of the Wellcome Laboratories of Tropical Medicine, where he has since organized chemotherapeutic research in the tropical field. In spite of the heavy administrative work of an army pathologist, Brigadier Boyd always found time to carry out his own particular research and has continued to do the same in his recent appointment. His most important work has dealt with the analysis of the multiplicity of antigens in the group of the dysentery bacilli, and with bacteriophages, particularly with the problems arising from the existence of symbiotic phages. In both these fields he has gained an international reputation. He was elected F.R.S. in 1951 and F.R.C.P. soon afterwards. It is fortunate that his new appointment will not prevent him from continuing his researches.

Dr. R. S. F. Hennessey, C.M.G.

Dr. R. S. F. Hennessey, who succeeds Brigadier Boyd, recently retired from the Colonial Medical Service, in which he has spent twenty-five years. He received his medical education at Trinity College, Dublin. Primarily a pathologist, he was employed as such by the Uganda Government from 1929 until 1944, and during most of that time he was lecturer in pathology and forensic medicine at Makerere College Medical School. From 1944 he served for three years with the Palestine Government, first as deputy director of medical services (laboratories) and later as deputy director of medical services (health). Thereafter he became assistant medical adviser to the Colonial Office in London and a member of the Colonial Medical Advisory Committee. His most recent appointment, during 1949–55, was as director of medical services, Uganda Government, in which capacity he was a member of several research committees. Dr. Hennessey thus takes with him to his new appointment a sound background of administrative experience and a wide knowledge of the outstanding problems of tropical medicine.

U.S. National Science Foundation

Dr. Alberto F. Thompson has been appointed head of the Office of Scientific Information of the U.S. National Science Foundation, and Mr. Clyde C. Hall, public information officer of the Foundation. Dr. Thompson goes to the foundation from the United States Atomic Energy Commission, where he has served for the past seven years as chief of technical information. He was in charge of the United States exhibit at the International Conference on the Peaceful Uses of Atomic Energy at Geneva. Before the War, he was an assistant professor of chemistry at the Massachusetts Institute of Technology, and earlier, a member of the faculty of the University of Minnesota. Mr. Hall was special placement representative in the Civil Service Commission, with major responsibility for the identification and placing of top management specialists in the Federal services. During the War, he served as assistant to the Director of Public Information for the Foreign Economic Administration and, earlier, as an information specialist with the U.S. Department of Agriculture. Before that he was a staff writer for the *National Geographic Magazine* and the Washington Bureau of the Associated Press.

Nuclear Energy Committee of the Institute of Metals

The Council of the Institute of Metals has appointed a Nuclear Energy Committee, with the object of stimulating interest in metallurgical problems arising in connexion with the industrial and scientific aspects of the use of nuclear energy. It is proposed that, among its activities, the new Committee shall arrange special meetings, discussions and symposia; invite the writing of papers or articles for publication; and arrange the publication of books or other literature to attain the objects for which it was formed. The Committee hopes to arrange an exhibition of nuclear energy products, of interest to metallurgists, in connexion with the Institute's Spring Meeting in April 1956. The constitution of the Committee is: Mr. G. L. Bailey (British Non-Ferrous Metals Research Association), Mr. E. W. Colbeck (Hadfield, Ltd.), Dr. Maurice Cook (Imperial Chemical Industries, Ltd., Metals Division), Dr. S. F. Dorey (Lloyd's Register of Shipping), Dr. H. M. Finniston (United Kingdom Atomic Energy Authority), Dr. Ivor Jenkins (The General Electric Co., Ltd.), Prof. A. G. Quarrell (University of Sheffield), Mr. L. Rotherham (United Kingdom Atomic Energy Authority), Mr. S. S. Smith (Imperial Chemical Industries, Ltd., Metals Division), and Major P. L. Teed (Vickers-Armstrongs (Aircraft), Ltd.). Anyone interested in the activities of the Committee should communicate with Lieut.-Colonel S. C. Guillan, Secretary, Institute of Metals, 4 Grosvenor Gardens, London, S.W.1.

An Electronic Computer for the Meteorological Office

This is the centenary year of the Meteorological Office, and through its long record meteorologists have been confronted with dynamical problems of such complexity that their solution has sometimes seemed beyond hope. In recent years, however, nothing less than a revolution has taken place. The analysis of regular upper-air observations has revealed much about the physical processes, and the invention of electronic computers has opened the way to numerical solutions to problems the analytical solutions of which are

prohibitively difficult. The staff of the Napier Shaw Research Laboratory of the Meteorological Office have already made a major contribution in this new advance and, confirming work in other countries, especially the United States and Sweden, have shown that weather maps, giving isobars and upper air contours, may be predicted for 24 hr. ahead by basic hydrodynamical methods and with promising accuracy. The numerical computations are extremely heavy and hitherto have been performed by arrangement on digital electronic computers available in London and Manchester. The requirement for a machine for the sole use of the Meteorological Office has, happily, now been recognized, and it is expected that one of most modern type will be installed at Dunstable within a year or so. Whatever may be the future success of weather prediction, this step may be taken as marking a turning point in the science of meteorology. Other problems, not only those of forecasting, will no doubt yield ground to an attack by quantitative methods, and the recent claims made by the director, Sir Graham Sutton, for meteorology as an exact science must more and more be realized.

Science Service

Science Service was founded in America in 1921 as a non-profit corporation with trustees nominated by the National Academy of Sciences, the National Research Council, the American Association for the Advancement of Science, the E. W. Scripps Estate, and the journalistic profession. In the original statement of aims its task was defined as "to take science to the people . . . to conduct, operate and furnish press service for the collection, preparation, distribution and sale of scientific matter and information, current events and fundamental conceptions or topics of interest to general readers, to newspapers, periodicals, journals, magazines, educational institutions and government". One of its developments has been the Science Clubs of America, an organization in which about 350,000 boys and girls are affiliated to fifteen thousand clubs in the United States and other countries. The clubs vary in size from three to seven hundred members, and programmes are planned to suit the age and science interests of the group. Each year two national events are held for members of Science Clubs of America. The annual National Science Fair was started in 1950 and is held each spring in a different city. The host city in 1956 is Oklahoma City and, in 1957, Los Angeles. The other national event was started in 1942 and, as the Annual National Science Talent Search, is held each year for seniors in high school who wish to compete for 11,000 dollars in Westinghouse Science Scholarships for their college education. The winners receive a free visit to Washington to attend the Science Talent Institute. All the brochures produced by Science Service are set out attractively and may be obtained from 1719 N. Street, N.W., Washington 6, D.C.

University of Birmingham

The University of Birmingham has conferred the title of reader in mathematical physics on Dr. R. H. Dalitz. The following have been appointed lecturers in the University: Dr. P. H. Rose (physics), S. W. Green (botany), Dr. J. S. M. Botterill (chemical engineering), J. W. R. Griffiths and J. C. Cluley (electrical engineering), and Dr. H. Y. Yeung (electron physics). The following have been appointed to fellowships: Dr. C. B. B. Bull (geophysics), Dr. A. M. Bolsterli and Dr. E. Vogt (mathematical physics), D. B. Idle (botany), Dr. R. A. James and S. Markowitz (physics), Dr. Eleanor H. Slifer (zoology), and Audrey Todd (chemistry).

Gifts have been made to the University as follows: to the Department of Chemistry, £550 from the Electrical Research Association, for one year in the first instance, for research on the fundamental characteristics of the oxidation of certain liquid hydrocarbons, and £700 rising to £850 over three years from S. C. Johnson and Co. for a research fellowship to be devoted to research on waxes; to the Department of Civil Engineering, two grants from the Highway Engineering Education Trust, one for establishing a lectureship in highway engineering for a period of three years and the other, amounting to £250 a year for two years, for the organization of a summer school to give advanced training in road construction, traffic engineering and highway soil mechanics; to the Department of Electrical Engineering, £800 from the Central Electricity Authority to continue research work on electrostatic precipitation of dust; to the Department of Chemical Engineering, £935 for one year from the Ministry of Supply for research on the low-temperature properties of esters, £600 a year for two years from Whiffen and Sons, Ltd., for research in liquid-phase corrosion, £3,675 over a period of three years from the United Kingdom Atomic Energy Authority for research on the characteristics of 'Spraypak', and £775 from the Ministry of Supply for research on the design of distillation towers; and to the Department of Physical Metallurgy, a total grant of £3,225 for three years from the United Kingdom Atomic Energy Authority for research on the properties of ionic crystals.

University of Leeds: Recent Gifts

The following gifts have been made to the University of Leeds: £1,000 from Glover and Main, Ltd., London, for North Block I (Fuel Science) of the University; 6,000 dollars from the Dow Chemical Co., Michigan, to the Department of Physical Chemistry for further research in paramagnetic resonance; £6,627 from the Wool Textile Research Council to the Department of Textile Industries for research under the direction of Prof. J. B. Speakman and £3,340 for 1955–56 for research under the direction of Prof. A. H. Nissan; £900 from the Wool Textile Research Council to the Department of Colour Chemistry and Dyeing for research; and £900 for 1955–56 from the Cement and Concrete Association to the Department of Civil Engineering for the salary of a lecturer.

National Institute of Sciences in India: Proceedings

The National Institute of Sciences in India, since its inception in 1935, has published regularly every year the *Proceedings of the National Institute of Sciences in India*, containing miscellaneous articles on physical and biological sciences, as well as important official proceedings of the Institute. The Council of the Institute has decided that, with effect from Vol. 21 (1955), the *Proceedings* are to be published in two parts—*A*, physical sciences; *B*, biological sciences—and they are to be issued bi-monthly in alternate months. In addition, the official proceedings are to be published in a "Year Book", an annual publication on the lines of the publications of the Royal Society. The volume and year numbers will be the same for the two parts of the *Proceedings*,

and each will contain its own title page, page numbering and index.

Science Masters' Association : Annual Meeting

The annual meeting of the Science Masters' Association will be held in the Royal College of Science, Imperial Institute Road, South Kensington, London, S.W.7, during December 28–30, the annual business meeting of the Association being on the last day. The president this year is Sir Edward Salisbury, director of the Royal Botanic Gardens, Kew, and he will deliver his presidential address in the Courtauld Hall, Queen Elizabeth College, on the evening of December 28, his subject being "Science and Education". Two special discussions will be held : one on December 30 at which science teaching in secondary modern schools will be considered ; and one on December 31 on first-year failures in the universities, which will be introduced by Dr. E. R. Roberts, of the Department of Inorganic Chemistry, Royal College of Science, and by Prof. F. A. Vick, of the Department of Physics, University College of North Staffordshire. On the morning of December 29 a lecture-demonstration of experiments with radioactive isotopes suitable for schools will be given by the members of the Radioactivity Subcommittee of the Association, and in the afternoon there will be a demonstration of ciné and cinémicrography in the teaching of natural history, given by K. A. Murch, of the Chichester High School for Boys. In the afternoon of December 30 the Science and Religion Group of the Association will hold a discussion, and in the evening Mr. T. D. Bourdillon, a member of the 1953 Everest Expedition, will lecture on the successful conquest of Mount Everest and on the use of oxygen during the climb, which was his special responsibility. As in the past, manufacturers and publishers who advertise in the *School Science Review* will exhibit at the meeting, and the usual exhibition by members of apparatus and teaching aids which they have found useful will also be held. In addition, special displays will be given in the Royal College of Science by the Civil Defence Department of the Home Office, and in Queen Elizabeth College by members of the Science Departments there. Further information can be obtained from the annual meeting secretary of the Association, W. H. Dowland, 93 Westbourne Road, West Hartlepool, Co. Durham.

British Institute of Radiology : Annual Congress

The annual congress and exhibition of the British Institute of Radiology will be held in Caxton Hall, Victoria Street, Westminster, London, S.W.1, during December 8–9. On the morning of the first day, an opening address will be given by Sir John Cockcroft, director of the Atomic Energy Research Establishment, on the future role of atomic energy in industry, science and medicine, and this will be followed in the afternoon by a symposium on the hazards from the increasing use of ionizing radiations, at which three papers will be read. In the evening Dr. Gwen Hilton (University College Hospital, London) will deliver the Mackenzie Davidson Memorial Lecture and will speak on the role of radioiodine in the treatment of carcinoma of the thyroid. The second day will be devoted to papers on the clinical applications of image intensification, and three symposia as follows : pulmonary fibrosis and collagen diseases of the lungs ; the influence of histological structure on radiosensitivity ; the introduction of the rad in radiotherapy practice. The fee for the congress is 1 guinea (10*s.* 6*d.* for members of the Institute). Further information can be obtained from the Honorary Secretary of the Institute at 32 Welbeck Street, London, W.1.

Christmas Lectures for School Children

A Christmas lecture for older school children, arranged by the Institution of Electrical Engineers, will be given at the Institution (Savoy Place, London, W.C.2) on December 29 at 3 p.m., and again on the following day, by Mr. R. Brooks, his subject being "Electric Traction". The lecture will outline the principal features and methods of operation of the different systems of electric traction now in use, particular reference being made to the equipment of the locomotives and motor coaches used on main-line and suburban services. The main principles of electric railway signalling will also be considered, together with a demonstration of full-size apparatus including searchlight-type signals. Admission to the lecture is free, and application for tickets, stating for which afternoon they are required, should be made to the Secretary of the Institution at the above address. The Royal Institution's Christmas lectures this year will be the 126th such "Course of six lectures adapted to a Juvenile Auditory". The course will be given by Prof. H. W. Melville at 3 p.m. on December 27, 29 and 31 and January 3, 5 and 7, his subject being "Big Molecules". The topics to be discussed at the six lectures will be, respectively, the making of big molecules, the size of a big molecule, rubber and the rubber-like state, man-made fibres, and plastics from big molecules (two lectures). For non-members of the Royal Institution, the fees for the course are : children (10–17 years) £1, adults £2. Application for tickets should be made to the Royal Institution, 21 Albemarle Street, London, W.1.

Announcements

Sir George Lloyd-Jacob, one of Her Majesty's Judges of the High Court of Justice, has accepted appointment as a Trustee of the Ciba Foundation for the Promotion of International Co-operation in Medical and Chemical Research.

The University of Sheffield has conferred the title of emeritus professor of engineering on Prof. H. W. Swift, and that of emeritus professor of glass technology on Prof. H. Moore, both of whom have recently retired from their respective chairs.

The Department of Scientific and Industrial Research has appointed Sir Walter Drummond, formerly chairman of the National Coal Board, and Prof. N. F. Mott, Cavendish professor of physics in the University of Cambridge, to be the Department's visitors to the Electrical Research Association, in succession to Dr. P. Dunsheath and Prof. J. T. Randall, whose period of service has been completed.

The Mond Nickel Co., Ltd., has appointed Dr. G. L. J. Bailey to be superintendent of its Platinum Metals Research Laboratory at Acton, London, N.W.10, in succession to Dr. E. C. Rhodes. Dr. Bailey, who is a graduate of the Imperial College of Science and Technology, London, was on the staff of the Admiralty Engineering Laboratory, West Drayton, throughout the Second World War, and since 1945 he has been a member of the British Non-Ferrous Metals Research Association, where he is at present deputy research manager.

CONFERENCE OF COMMONWEALTH SURVEY OFFICERS

THE Commonwealth Survey Officers' Conference was held in London and Cambridge during August 15–26. It was opened by Lord Lloyd, Under-Secretary of State for the Colonies, in the Department of Geography at Cambridge, and was attended by about two hundred representatives of Home and Commonwealth surveying departments and allied interests, including the directors of survey or surveyors-general of nearly all the Commonwealth territories, with observers from the United States of America, France, Holland, Belgium, Ireland and the Sudan. Between August 15 and 23, twenty-six papers were read on a wide variety of technical and administrative subjects, after which the Conference moved to London for visits to neighbouring government survey establishments. Brigadier M. Hotine, director of Colonial surveys, was president of the Conference. Delegates were mostly accommodated in Pembroke and Downing Colleges, a very convenient arrangement. A valuable adjunct was an exhibition of surveying instruments by eighteen British manufacturers.

Of the papers read, eight dealt with administration, education and land registration; three with the design of new instruments; eight with the routine of field surveying, nearly all from the point of view of air survey; three with the geodetic framework; one with variations of sea-level; and three with electronic aids to surveying. The list of titles might suggest that an aircraft and a box-full of electronics is now a good substitute for hard work on the ground; but in one of the discussions it was well agreed that in most circumstances the best map could still only be made by a fairly equal combination of both processes. The following notes give a short summary of the papers most likely to be of interest to readers of *Nature*.

In a paper on the use of automatic plotting machines for 1/1,250 air survey, Major D. V. Hutchinson described the combination of air and ground survey now being used for the city survey of Oxford, which will probably serve as a model for similar plans throughout Great Britain. The air-photo plotting machine requires less dense ground control than the old method of chain survey on the ground, but does not, of course, provide a map which can be accepted without ground verification and amplification. Clear diagrams of a sample plan show the amount of detail (about half) which is confidently identified on the photograph and plotted by the machine, and how much is left to be verified and amplified by the ground surveyor: at this scale, buildings with overhanging eaves come into the latter category. The out-turn of the ground surveyor has been increased from 0·28 sq. km. a month without preliminary air survey to 0·48 sq. km. with it. Total costs, including that of flying, are substantially lower than when ground survey alone is employed.

Prof. E. H. Thompson's new air-photo automatic plotting machine, of which the first model has just been completed by Messrs. Hilger and Watts, was on view and was the subject of a paper.

A paper by Major I. E. Sleep analysed the accuracy of a 1 : 25,000 map made by multiplex equipment from 1/90,000 vertical photography, supplemented by 1/30,000 obliques, taken from an aircraft at 45,000 ft., an altitude at which flying may be convenient in time of war. With ground control-points about 11 miles apart, errors of height were found to average 16 ft. and of position 12 metres, a standard which is adequate for a military 1/25,000 map with 20-metre contours, and which is indeed remarkable considering the scale of photography and scarcity of control. A typical 1/25,000 map sheet made in this way accompanies the paper, with the Ordnance Survey version (errorless in plan, but not in contouring) printed in grey, and the air-survey version in brown.

Surveyors will be interested to learn from a paper by E. W. Taylor that the designers of the new small Tavistock theodolite have changed over to the coincidence system of reading, and that both circles are now read in a single eyepiece alongside the main telescope. In the geodetic model, a similar coincidence system is also being adopted; but the separate micrometers and eyepieces for the horizontal and vertical circles are retained.

Commander W. I. Farquharson read a paper containing interesting descriptions of tidal bores, seiches, surges and secular changes of sea-level. From British tidal records, which are regrettably scanty, he has analysed the secular rises of sea-level during 1835–95, 1895–1925 and 1925–50, and finds them to be zero, 0·4 ft. per century and 0·5 ft. per century, respectively. He quotes rises of 0·4 and 1·2 ft. per century for the two later periods from American records at Halifax, New York and Baltimore, and a similar value from Holland. He suggests that port engineers should be prepared for a further rise of 1 ft. during the coming century.

Capt. C. C. Lowry's paper on radio aids to hydrographic surveying described the uses and limitations of radio distance, depth and direction measuring apparatus as applied to hydrographic survey, and a paper by C. Powell gave details of the construction, use and accuracy of the Decca equipment.

A paper by D. L. Mills, of the United States Army Map Service, described the completion of the African Arc of the 30th meridian. This great piece of geodetic triangulation, which was begun about seventy years ago, has been finally completed since 1952 by a party from the U.S. Army Map Service observing the section between latitudes 1° and 10° north. This section includes 300 miles of the flat Sudd region, where triangulation is difficult and has only been made possible by using 100-ft. portable Bilby steel towers. Three base-lines were measured by the Sudan Survey Department, and Laplace azimuth stations were observed at appropriate intervals.

One paragraph in this report answers a question which has often troubled surveyors, namely, what happens when a Bilby tower collapses. One such accident occurred, a tower collapsing with five men at the 100-ft. level. One broke a bone in his foot and the others were at work again after a day's rest. The ground was hard-baked clay. The Sudanese are evidently resilient.

The completion of the African arc, coupled with the radar connexion recently observed between Crete and Egypt, makes it possible (if desired) to compute

South African triangulation in terms of the European Datum. Russian triangulation, known to exist but not published, could carry the same datum to Vladivostok, and a gap of 1,000 miles in Persia is all that prevents it extending to Singapore. Radar measures, of geodetic accuracy, between Europe, Greenland and America will link the European and American datums within a few years, and triangulation from the latter already extends well into South America. The days of separate, isolated national surveys are passing.

A paper by H. A. Stamers Smith gave a useful summary of the present state of geodetic triangulation in Nigeria, where primary traverse has in places been very successfully used as a substitute for triangulation. The misclosures of ten such traverses are given, averaging about 1 in 100,000 in distances of 100–200 miles.

A paper by Col. R. C. A. Edge described work done by the Ordnance Survey with electronic computing. Traverse and levelling computations are a heavy item in the ordinary work, but, varying much in detail, require a high proportion of 'arranging' for pure computation. They are therefore more suited to punch-card methods using a small calculator (such as the *I.B.M.* 626) than to a large storage computer. An *I.B.M.* 626 has recently been installed at the Ordnance Survey. Photogrammetric computations, another heavy item, are more uniform but mathematically more complex. They are suitable for a large computer, and for some time they have been programmed for, and successfully computed by, *ACE*. They are now being programmed for *DEUCE*.

Space does not admit mention of the remaining papers, but all are of value in their own spheres. These Survey Conferences are held at four-yearly intervals, and provide a most welcome opportunity for surveyors from all over the Commonwealth to exchange ideas and to renew friendships. The papers presented at previous conferences, and the resulting discussions, have been published by H.M. Stationery Office, and it is hoped that a 1955 volume will soon be published also.

G. Bomford

INTERNATIONAL BIOMETRIC SYMPOSIUM IN BRAZIL

THE Biometric Society, which now forms the Biometric Section of the International Union of Biological Sciences, is endeavouring to stimulate the teaching and use of good statistical practices in biology in many parts of the world. As part of this programme, an international biometric symposium was held in Campinas, Brazil, during July 4–9. The University of São Paulo was host to the symposium, and a committee of the University Seminario de Estatistica, with Dr. C. G. Fraga (Institute of Agronomy, Campinas) as secretary, handled local arrangements; financial assistance was received from ten different governmental and non-governmental organizations. The ninety-eight statisticians and biologists attending included sixty-two from Brazil, eight from other Latin American countries, fourteen from the United States, five from Great Britain, seven from other European countries, and two from Asia. The foreign visitors will long remember the hospitality of their Brazilian hosts.

The president of the Society, Prof. W. G. Cochran (Johns Hopkins University), took as the subject of his address the 1954 trial of the Salk poliomyelitis vaccine in the United States, focusing attention on the design of the investigation rather than upon the outcome. He said that in 'placebo areas' children who received the vaccine were matched by controls receiving a similar injection of a placebo, while in other parts of the country the only controls were younger and older uninoculated children. The difference between case-rates in inoculated and control children was apparently greater in the placebo areas. His suggested explanation was that natural immunity was lower in the homes of children whose parents consented to their inclusion in the trial, a consent necessary in the placebo areas for all participators but elsewhere only for the inoculated.

In the afternoon, Sir Ronald Fisher (University of Cambridge) described the contribution of biometry to plant improvement, with special reference to experimental design, the analysis of genetic models involving large numbers of Mendelian factors that cannot be individually identified, and classical Mendelian genetics. Prof. E. R. Dempster (University of California) then discussed genetic models in animal breeding. Shorter papers on biometrical genetics by Prof. F. G. Brieger (Agricultural College, Piracicaba) and Dr. H. Kalmus (University College, London) related to autogamic populations and heterotic genes in plant genetics and to cyclomorphosis, respectively.

Two sessions on experimental design were held on July 5. Dr. S. C. Pearce (East Malling Research Station) showed that difficulties of adequate replication with perennial crops restrict the experimenter to very simple designs. A compensation is that relationships between many concomitant measurements can be studied. He illustrated the balancing of treatments so that one set of trees can be used for two or more consecutive investigations. E. Amaral (Institute of Agronomy, Pelotas) discussed missing yields and other problems in the analysis of sugar-cane experiments. The design and analysis of coffee experiments at the Campinas Institute of Agronomy were reviewed by Dr. A. Conagin and Dr. Fraga, who showed how modern design had improved the quality of information obtainable in research on this crop. In a more theoretical paper, Dr. F. Pimentel Gomes (University of São Paulo) spoke of the use of the Mitscherlich equation in analysing fertilizer experiments on sugar-cane. In the afternoon, Miss G. M. Cox (University of North Carolina) surveyed recent work in the theory of experimental design; her paper was of particular interest for its mention of special designs for estimating optimal conditions, including the new category of rotatable designs. Dr. W. J. Youden (Bureau of Standards, Washington, D.C.) then gave a graphic account of how types of design developed by biologists are now assisting research on physical problems. With remarkable clarity and humour, he illustrated the adaptation of designs to situations in which the block size is predetermined; or, at most, two replications are wanted; or some treatments are to be chosen after part of the experiment was complete; or in which instrumental drift is a serious factor. On the following day, during a visit to the Agricultural College at Piracicaba, members held a discussion on experimental design for perennial crops.

On July 7, Prof. P. G. Homeyer (Iowa State College) summarized experience on sources of variation in feeding experiments with chicken, cattle and pigs, and its use in designing new experiments; he emphasized particularly the danger of neglecting variation between pens. Dr. G. L. Rocha (Brazil) and Dr. G. O. Mott (Purdue University) reviewed the technique and interpretation of pasture experiments in Brazil and the United States, respectively, after which Prof. A. Linder (University of Geneva) described an experiment on the grazing times of cows permitted to choose between differently treated plots. In the afternoon session, on sampling techniques, Dr. M. H. Hansen (United States Bureau of the Census) discussed the control of errors of response in sample surveys. Recent work on the theory and practice of estimating fish catches around the Indian coast was then described by Dr. P. V. Sukhatme (Food and Agriculture Organization). Prof. J. Nieto de Pascual (Bureau of Statistics, Mexico) described a survey of national morbidity in Mexico, and Prof. W. L. Stevens (University of São Paulo) presented an account of recent work on the sample estimation of the coffee harvest, a problem complicated by a biennial cycle of bearing. The session ended with a paper from Dr. E. Cansado (Interamerican Statistical Centre, Santiago, Chile) on new proofs of theorems on sampling from a finite population.

After a morning visit to the State Institute of Agronomy at Campinas, the afternoon of July 8 was given to papers on bioassay. Dr. C. I. Bliss (Connecticut Agricultural Experiment Station) showed general methods for obtaining confidence intervals for estimates of relative potency, and then illustrated how these could be adapted to various assay techniques in the "U.S. Pharmacopeia". Dr. D. J. Finney (University of Aberdeen) presented five models relating to correlations of residual error and residual effects of previous doses in cross-over and single-subject assays, and explained the statistical analysis appropriate to various designs. Dr. P. Mello Freire (Institute of Biology, São Paulo) described the analysis and interpretation of three 'complement fixation' assays. Finally, several questions on bio-assay, with special reference to the assay of snake venom, were submitted to a panel for discussion.

The first of four papers on medical statistics on July 9, that by Dr. J. O. Irwin (London School of Hygiene and Tropical Medicine), related to an experiment conducted in Singapore with human subjects to assess the influence of air movement, temperature, humidity, clothing and other factors on sweat-rate and other physiological measurements. An interesting application of covariance analysis was shown by Dr. J. N. Manceau (Public Health Service, Pará) in his account of a comparison between two antihelminthics for the control of nematode parasites in seventy-four Belém children. Dr. A. E. Brandt (United States Atomic Energy Commission) described briefly an investigation into the clinical effects of two different very high-voltage sources proposed for the treatment of cancers that are frequently incurable by conventional radiotherapy; he then considered the problems of performing a clinical trial on one type of cancer, with adequate random allocation of patients to the two treatments yet within the limits imposed by medical ethics. The final paper, by Prof. A. Vessereau (Sorbonne), consisted of an interesting application of discriminant function analysis to the diagnosis of medical and surgical cases of jaundice from electrophoretic analysis of serum proteins.

One of the most satisfactory features of the symposium was the lively discussion that followed almost every paper: the variety of questions and comments, and the number of people taking part, proved that the holding of the symposium was fully justified. Moreover, Brazilian science will continue to benefit from this venture, for it provided the impetus for the immediate formation of a Brazilian Region of the Biometric Society (which already has more than fifty members) and also of a Brazilian Genetical Society.

D. J. Finney

DESIGN OF ELECTRONIC DEVICES FOR PRODUCTION

A SUMMER school on the role of the trained scientist in the design of electronic devices for production was held at the Services Electronics Research Laboratories in Baldock and Harlow during September 20–23. More than sixty university and technical-college professors and lecturers attended. Accommodation was provided by Emmanuel College, Cambridge, and transport to and from the Laboratories was by motor coach.

The first speaker was Mr. A. J. Young (English Electric Valve Co.), who surveyed the recent developments in special valves. He directed attention particularly to one of the functions of management in the device industry, namely, to decide to pursue a particular line of development. The scientific judgment required to execute this function to the best advantage is an increasing need in the industry, and it should be the object of the universities and technical colleges to bring forward scientists having good understanding of their subject and, as a result, good judgment regarding device development. The next speaker was Mr. D. S. G. Lewis (officer in charge of the extension of the Services Electronics Research Laboratory at Harlow), whose subject was magnetrons, and he gave a résumé of the problems facing the designer after fifteen years of development. In particular, he directed attention to the complicated nature of the device, in which the variation of any one parameter alters the effect of every other one. Mr. Lewis sketched the problems arising in the manufacture of magnetrons, giving special attention to the close limits imposed at very high frequencies. The final speaker on the first day was Dr. J. M. Dodds (Metropolitan-Vickers Electrical Co., Ltd.), his subject being high-power klystrons. He explained why the klystron is preferred to the magnetron for certain purposes, and described the design and manufacture of a klystron suitable for driving a very high-power linear accelerator. During this first day examples of modern valves were exhibited, many valve firms contributing samples of their latest designs. In the afternoon, Dr. H. A. H. Boot, one of the inventors of the multi-cavity magnetron, supervised a demonstration of micro-wave valve-making techniques.

The second day of the school opened with a discussion of methods of generation of millimetre waves, given by Mr. P. O. Hawkins (Services Electronics Research Laboratory). Mr. Hawkins, in addition to surveying the field, gave special attention to a floating drift tube klystron capable of delivering 10–20 W. at a wave-length of 8 mm. He was followed by Dr.

R. Q. Twiss (also of the Services Electronics Research Laboratory), who illustrated forward thinking by discussing the prospects of developing and detecting sub-millimetre waves; he gave special attention to the use of electrons travelling at approximately the speed of light. Mr. G. Knott (Mullard Research Laboratories) described the action of travelling-wave amplifiers and of backward-wave oscillators. In the first part of his discourse he referred particularly to the problem of noise in signal amplifiers and to the yet unsolved problems of mechanical design; in the second part he sketched the theory of backward-wave valves and described results achieved at a wave-length of about 2 cm. which may lead to a new generation of low-power micro-wave oscillators.

The school then turned its attention to the important subject of reliability in electronic devices, and on this subject an address was given by Mr. E. G. Rowe (Standard Telephones and Cables, Ltd.). Mr. Rowe explained what steps had been taken since 1945 to improve the general reliability of valves and described the problems which still remained to be solved. He was followed by Mr. G. H. Metson (Research Station of the General Post Office), who dealt specifically with the problem of designing valves for lives of the order of a hundred thousand hours (more than eleven years) and gave a scientific explanation of the factors determining long life in cathodes. In the morning a demonstration was given of millimetre-wave generators, and in the afternoon Mr. P. Gurnell superintended a demonstration of receiving-valve manufacturing techniques. The day closed with a lively discussion of how best to teach at university standard so as to obtain industrial scientists having a real understanding of scientific problems.

On September 22 the school transferred to Harlow, where it was addressed by Dr. D. A. Wright (Research Laboratories of the General Electric Co., Ltd.) and later by Mr. C. P. Lea-Wilson (Harlow) on the subject of thermionic cathodes. Dr. Wright described all types of thermionic emitter, dealing particularly with the limitations of the oxide-coated cathode, while Mr. Lea-Wilson paid special attention to high-temperature sintered cathodes used in magnetrons and high-power klystrons. Mr. J. Pollard (Services Electronics Research Laboratory) then discussed methods of obtaining high vacua and explained that the needs of the valve engineer are not yet adequately met either as regards speed of pumping or as regards ultimate vacua. In the afternoon Mr. J. E. Stanworth (British Thomson-Houston Research Laboratory) described the problems arising in the manufacture of glass and ceramic valve envelopes. In the second part of his address he dealt specifically with some of the new and interesting glasses developed recently. In the evening Mr. A. Monk (Harlow) supervised a demonstration of the use of new materials in valve making.

The final day of the school was devoted to problems arising in connexion with solid-state devices. The introductory discourse was given by Mr. I. M. Ross (Services Electronics Research Laboratory), who surveyed the whole field, giving examples of entirely new solid-state devices which have come to light during the past few years. He was followed by Mr. E. H. Cooke-Yarborough (Atomic Energy Research Establishment), who described the properties of transistors and some of the uses to which they are being put. He emphasized that the transistor is a new type of electronic device in many ways utterly dissimilar to a valve and demonstrated some of the surprising circuits in which transistors can be used. Mr. E. B. Callick (Royal Naval Scientific Service) then discussed the problems encountered in manufacturing transistors and emphasized that these represent an entirely new development in manufacturing methods, where extremely minute traces of impurity are of primary importance and where the physical magnitudes involved are very small indeed. In the afternoon Mr. A. F. H. Thomson (Harlow) described the various types of memory or storage devices which are coming into use, taking the view that magnetic materials will play an ever-increasing part in this field. He was followed by Mr. P. E. Trier (Mullard Research Laboratories), who dealt more specifically with magnetic switching devices. He showed how these can be built up into a full-scale computer and gave special attention to the difficult scientific problems which have yet to be solved in connexion with the use of ferro-magnetic materials and ferrites. During the afternoon a demonstration was given of solid-state devices in use in various ways.

In the short time which remained for discussion it was suggested that the proceedings of the school should be made available, at least in an abridged form, and it is hoped to publish these in the *Journal of Electronics* early next year. J. Thomson

ANIMAL MOVEMENT

During the British Association meeting at Bristol, Sections D (Zoology) and I (Physiology) held a joint session on September 6, at which the subject of "Animal Movement" was discussed. The first paper was by M. A. Sleigh, speaking on ciliary movement, and in it he gave an account of his own work on the movement of the peristomial cilia of *Stentor*. He suggested that the frequency of beat is a measure of the mechanical activity and that wave velocity is a measure of the metachronal co-ordination. Experiments have shown that the beat frequency and wave velocity have different temperature coefficients. Also it can be demonstrated that changes in the viscosity of the medium and in the viscosity of the protoplasm affect only the frequency and not the wave velocity, while the wave velocity can be substantially altered by digitoxin without appreciably affecting the frequency. The conclusion to be drawn is therefore that the two parts of the system are separate and have very different properties. Mr. Sleigh holds the view that the frequency might be maintained by the first cilium in a row acting as a pace-maker, while the excitation pathway of the wave velocity might be neuroid.

The second paper, by Dr. T. Weis-Fogh, was on the flapping flight of insects, and was illustrated by a slow-motion film on the flight of the desert locust. He described the wing movements in detail, and explained how the film was made and how his co-worker, Dr. Martin Jensen, had used it for estimating the changes both in magnitude and direction of the aerodynamic forces during the beat cycle. It has become clear that the wing cycle can be treated as a series of stationary flow situations and that the torques set up at the wing base can be estimated. When these torques are considered in detail, it is possible to subdivide them into aerodynamic and inertial torques, and to relate them to the torque-producing components within the body. From this

analysis, it is evident that the inertial torques are almost balanced by elastic torques arising from deformations of the body cuticle. When the various torques are summed for different phases of the beat, the result equals the torque which the flight muscles must produce. It appears that some two-thirds of the work is being expended against aerodynamic forces, and that the mechanical efficiency of the muscle system is 17–18 per cent.

The third speaker, Dr. R. H. J. Brown, discussed the flapping flight of birds. He pointed out that there are several different types of flapping movements, and that these are found either in different birds or in the same bird in different situations. The various types of flapping cycle were illustrated by lantern slides, and the functional differences between the various phases were described. It is clear that whereas the down-strokes are almost the same for all birds, the up-strokes may be entirely different both in path and function. These various types of up-stroke are found, with few exceptions, to be related to the size of the bird. The small birds up to the size of a pigeon usually fold their wings entirely during the up-stroke in slow flight. The pigeon and birds of similar size use a propulsive up-stroke in slow flight and a passive one in fast flight; and in the large species the up-stroke is passive except perhaps just before landing. Individual flight habit makes the transition point from one type to the other indefinite. It can be shown that these differences are to be expected from considerations of muscle weight, wing area and wing mass (inertia). While the qualitative picture is fairly clear, there is little information from which any quantitative estimates of aerodynamic or overall efficiency can be made.

The last paper was given by Dr. O. C. J. Lippold, who considered fatigue in human voluntary muscle. He suggested that the fatigue may be attributable to one or more of four factors: first, the voluntary drive may be diminished; secondly, the nerve conduction may be impaired; thirdly, the neuromuscular junction may be fatigued; and finally, the contractile mechanism may itself become weaker. It is probable that three of these factors are responsible for the diminished activity in fatigue, and that the main effect is due to one or other in different circumstances. For example, prolonged work, not of a strenuous nature, undoubtedly gives rise to central fatigue; but if a strong enough motive arises, the muscle activity can be restored to the unfatigued level. To what extent the occurrence of a neuromuscular block is important has not been settled, although experiments described by Dr. Lippold lead him to believe that such fatigue does not occur. He also holds the view that fatigue of the neural pathway is not a factor. On the other hand, under conditions of strenuous exercise it is clear that the fatigue can be predominantly muscular, and that the onset of fatigue is accelerated and the recovery delayed by impairment of the blood supply to the muscle. Finally, Dr. Lippold suggested methods by which the blood supply to the muscle might be increased, and thus postpone the onset of fatigue.

During the short discussion which followed, Mr. Sleigh was questioned on his suggestion of a neuroid control in ciliary movement and pointed to the structures associated with cilia as supporting his views, and then Dr. Weis-Fogh explained some of his estimates of internal work and power losses in locust flight.

STANDARDS IN BRITISH INDUSTRY

IN two recent broadsheets (Nos. 381 and 382, July 11, 1955) Political and Economic Planning reviews the various types of standards in use in British industry, examines the arguments for and against their use and scrutinizes the work of the British Standards Institution; in particular, the method of preparing standards and the extent to which they are adopted are considered, and whether the Institution, as at present constituted, is capable of doing justice to its growing responsibilities at home and overseas.

Industrial standards fall into two broad categories: functional standards, or the tools used in doing a job; and dimensional standards which, by laying down dimensions and tolerances for finished and semi-finished goods, secure interchangeability and simplification. Frequently there are combinations of both types. Britain's leadership in aircraft design has been helped considerably by the many standards relating to production, drawing-office practice and engineering construction, and objections to the use of standards in industry are often based on misconceptions of what standards imply. The first of these two broadsheets indicates the vital contribution which standardization can make to efficiency in industry by cutting out waste of machine-time, materials, power and labour, and also the importance of standards in foreign markets. Many problems are involved in the formulation of standards both technically sound and commercially practicable, and the resolution of these difficulties in Great Britain falls to the British Standards Institution, which is the subject of the second broadsheet.

Essentially the British Standards Institution is a co-ordinating body bringing together the technical knowledge of those engaged in industry, professional associations, technical colleges, universities and government departments, and the practical and commercial knowledge of producers, distributors and consumers in an endeavour to produce standards acceptable to all interests concerned. Until 1931, its activities were mainly confined to the engineering industry; but in that year a Supplemental Royal Charter enlarged its terms of reference to user industry as a whole and its name was changed from British Engineering Standards Association to British Standards Institution. By 1939 it had grown to something like its present size and published nearly a thousand standards for a wide range of engineering and chemical, building and like materials. It has always worked for the promotion of British standards abroad, and continues to take an active part in the movement for internationally accepted standards. Between the two World Wars it became concerned with the standards since known as Codes of Practice, of which more than a hundred and fifty have been issued, ranging from the planning of buildings for ensuring adequate sunlight to the use of electronic valves.

The broadsheet suggests that the Institution could take a wider view of the whole question of standards, and that its work at home should follow some order of priority more closely related to the productive efficiency of the country as a whole. Closer co-operation between the Institution and the British Productivity Council in an effort to introduce standards, where they are likely to be of most benefit, is

suggested; and with the growing adoption of standards overseas, the Institution must turn its attention increasingly to securing international agreement, aligning British standards to the requirements of overseas customers, and promoting British Standards in other countries. Some expansion of the publicity and information services of the Institution is desirable, and surveys are suggested which could indicate an order of priorities by showing where its work is most and least effective, particularly with regard to the smaller firm. The need for a solid financial basis is emphasized. The government contribution for 1955–56 should be of the order of £120,000; but the present arrangements terminate after 1956–57, and it is pointed out that it is important that the Institution's income must be assured for a sufficient period ahead to allow it to plan with confidence, possibly by means of a quinquennial government grant and equivalent longer-term subscriptions from industry.

THE POLYPEPTIDE CHAIN CONFIGURATION OF COLLAGEN

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THE complete determination of the structure of collagen presents two major problems: first, that of discovering the configuration of the individual polypeptide chains by the application of X-ray diffraction data; second, the understanding and significance of the larger-scale structure seen in the electron microscope. We reserve a discussion of the larger structure and its relation to that of the individual chains for a later communication. This communication deals with the polypeptide configuration and is an elaboration of earlier remarks[1,2] on the relationship between the structures of poly-L-proline and of collagen.

A consideration, in fact, of the recent work on this synthetic polymer[1,2] and on collagen itself[3–6] has clarified the principles on which the structure of collagen must be based, and shows that an approximate solution can now be arrived at with reasonable certainty. The determination of the exact atomic positions within a single chain will require a much closer study and may ultimately rest on the possibility of an improvement in the experimental data.

The main features of the collagen polypeptide chain configuration are, we think, indicated by the joint consideration of the following: (1) the high-angle X-ray diffraction pattern; (2) the amino-acid composition and sequence analysis; (3) the structure of poly-L-proline.

The configuration must also account for physical properties such as density and infra-red absorption.

High-Angle X-Ray Diffraction Pattern

The general features of the high-angle X-ray pattern are consistent with a system of hexagonally packed rods, 12 A. apart in dry material, the separation increasing with hydration. Each rod contains a set of atoms, which may be one or more amino-acid residues, repeated at axial intervals of 2·86 A. in the unstretched material. On stretching the fibre the axial repeat increases to 3·1 A. or more, and the improved X-ray pattern suggests that the atom sets are repeated on a helical structure. From the X-ray spacings, the dimensions of possible alternative helical systems can be derived[3–5]. Theoretically, a very large number of helical systems can be devised which fit the observed high-angle X-ray diagram, but it seemed to us that the actual model would be one of a comparatively small number of simple alternatives[3–6], namely: (*a*) a single helix with a period of 28·6 A., containing either three or seven turns and ten atom sets; (*b*) two concentric helices related by a diad axis, each helix repeating exactly after 57·2 A. with three or seven turns and twenty atom sets; (*c*) three concentric helices related by a triad axis, each helix repeating exactly every 85·8 A. Each chain would contain thirty atom sets and three or seven turns in its repeat. For three turns, since the atom sets and turns are both multiples of 3, there would be a complete repeat after 28·6 A.

Examination of the general form of the intensity distribution to be expected from such systems[5,6,9] and comparison with the observed high-angle X-ray pattern shows that, in collagen, the single helical form (*a*) is the most likely, as illustrated in Fig. 1. In the following discussion, this form is assumed to be correct.

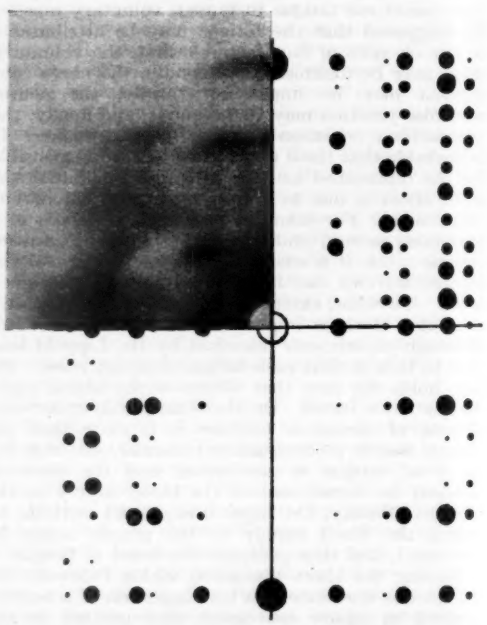

Fig. 1. High-angle X-ray diffraction diagram of stretched collagen alongside the calculated diffraction patterns of point atoms lying on helices of radius 3 A. The pitches correspond to the cases (*a*), (*b*) and (*c*) of the text as follows: top right, case (*a*); bottom left, case (*b*); bottom right, case (*c*)

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The measured density of dry collagen fibres shows that there are probably three amino-acid residues in the 2·86 A. atom set. The single-helix form (*a*) could then itself arise in one of several probable alternative ways: (i) a single polypeptide chain with three residues in 2·86 A.; (ii) a unit of three similar parallel chains, each with one residue in 2·86 A.; (iii) a unit of three chains, each with one residue in 2·86 A., so related that the complete system is specified by a single helical array of the dimensions of (*a*).

We have investigated a large number of structures of type (i), but have found none to be satisfactory. The difficulties of joining straight non-integral helices, as in (ii), by an adequate number of hydrogen bonds, have already been pointed out[3–6]. It is possible that these difficulties may be overcome if, instead of a system of three straight helices, we have a compound system of coiled coils, corresponding to case (iii).

The intensity distribution given by a compound helical system involving several single helices wound helically around one another is, as pointed out by Crick[10], more complex than for the simple cases, and it is not easy to formulate by inspection all the combinations of minor and major helix pitches which would produce a given intensity distribution. A particular case, however, is suggested by the poly-L-proline and collagen data. In this, three chains, each with three residues per turn relative to the major helix, slowly coil around each other so that the number of residues per turn, relative to the common axis, becomes 3¼. Such a system may be shown to correspond to case (iii), and is, therefore, of a form considered favourable from the analysis of the X-ray diagram. Further, the number of residues per turn in each chain relative to the other chains in the unit is integral, so that a regular form of hydrogen bonding is feasible.

Of the models proposed for the structure of collagen, only three[11–13] put forward since the helical analysis of the X-ray diagram was first described[3,7] have the required basic dimensions. The first two of these involve *cis* residues, which seems very unlikely in view of the infra-red spectrum of collagen[14,15] and of the structures of other peptides, particularly poly-L-proline; the third, that of Ramachandran and Kartha[13], is discussed below.

Amino-Acid Composition and Sequence Analysis

Although the complete amino-acid sequence in collagen has not yet been determined, some sequence studies have been made[16,17] and we can reach certain tentative conclusions. Examination of the small peptides which have been identified suggests that, if there is a repeated short sequence, it is not a rigid one (or is not maintained throughout the protein material), but is rather of the form $—R_1—R_2—R_3—$, where R_1 may be proline, R_2 may be hydroxyproline, and R_3 is, probably, nearly always glycine. The most interesting result is the isolation of the peptides glycyl prolyl hydroxyprolyl glycine and glycyl prolyl hydroxyproline. All three residues occur in sequences other than the above, with the exception that hydroxyproline always appears to be followed by glycine. Although glycine is about one-third of the total number of residues, proline is only one-eighth and hydroxyproline one-tenth in bovine collagen[18], so that at most the sequence —prolyl—hydroxyprolyl—glycyl— could only account for about one-third of the total structure. While glycine, the other non-polar residue, and the polar residues each account for about one-third of the total, the evidence does not suggest a rigid (—non-polar—polar—glycine—) sequence.

It may be that the sequence —prolyl—hydroxyprolyl—glycyl—, or a similar sequence, is largely concentrated in one of the three chains of a structure such as that discussed above, so conferring on this chain a special configuration. The other two chains can be pictured as crystallizing about this one. In any event, it is probable that a high proportion of these residues inserted into the α-helix[19] or β-pleated sheet[20] would make these structures unstable as a result of distortion and the reduced number of possible hydrogen bonds, and that a distinct type of chain might be the most stable. In the limit, a structure consisting entirely of proline residues might be expected to take up such a new configuration; this was in fact found to be the case.

Structure of Poly-L-Proline

An X-ray study of poly-L-proline[1,2], synthesized recently by Berger, Kurtz and Katchalski[21], shows the polymer to have a helical structure in which the amino-acid residues are related by an exact three-fold screw axis. The residue repeat along this axis is 3·12 A., and the similarity of this spacing to that of stretched collagen suggests strongly that the two chains have very similar configurations.

Poly-L-proline, like collagen, has a high specific *lævo* optical rotation[21]. It has been suggested that this rotation in collagen is due to the special type of helical configuration[22]. A left-handed sense is imposed sterically on the poly-L-proline chain, and it seems highly probable that the minor helices of a three-chain collagen structure would also be left-handed.

We now wish to consider ways in which three poly-L-proline type chains may be bonded together to form this compound system. The work described below was briefly referred to at the Third International Congress of Biochemistry, Brussels, 1955.

If one in three of the residues of the threefold chain has a secondary amide-group, then three chains can be imagined to be joined together by hydrogen bonds in two simple ways corresponding to the two trigonal positions in the poly-L-proline structure. In either system the individual left-handed chains are themselves related by a threefold left-handed screw. Looking in the —αC—NH—CO—αC— direction of the chains and taking the NH . . . O direction as the direction of hydrogen bonds, then the latter are alternately pointing clockwise or anti-clockwise. When the side-chain interactions are taken into consideration, the following results emerge. In the anti-clockwise system there are a number of short van der Waals contacts which would force the chains to take up a non-integral screw configuration. If a second proline ring is replaced by any side-chain, *R*, in the sequence —glycyl—*R*—prolyl—, there is a single short contact, which would cause distortion, whereas if two residues in three are glycine, there are no short contacts. In all cases the system might be stabilized with a three-and-a-third-fold screw-axis by additional hydrogen bonds. In the clockwise system, there do not appear to be any seriously short contacts, nor can such additional hydrogen bonds be readily made, and, although a three-and-a-third-fold structure can be built, there seems little reason for the chains to depart from the exact three-fold con-

figuration. A preliminary investigation suggests that in the anti-clockwise arrangement there may be two simple ways of making these extra hydrogen bonds: first, by replacement of a second proline in the three-residue sequence by another secondary amide residue, so that by slight distortion of the chains a second set of NH . . . O hydrogen bonds can be formed between peptide backbones; or second, by using the polar groups of side chains. One of the remaining proline residues could be replaced by hydroxyproline or by other hydroxy, acidic or basic residues, which might allow formation of an OH . . . O or NH . . . O bridge to an unbonded carbonyl group. The work of Gustavson[23], which shows a correlation between thermal stability and hydroxyproline content of various collagens, is in favour of an O—H . . . O hydrogen bond, in the case of hydroxyproline at least, although there does not at the moment appear to be any correlation with the proportion of other polar residues.

While this work was in progress, we became aware that very similar lines of thought were being pursued by other workers in the field. Ramachandran and Kartha[13] have been the first to propose a structure, having the superhelical configuration described above, in which the basic NH . . . O bonding is in an anti-clockwise direction with the structure additionally stabilized in the first of the ways mentioned above. A rigid —prolyl—glycyl—*R*— sequence is required, so that the structure as it stands cannot accommodate a number of known sequences, particularly —prolyl—hydroxyprolyl—. The hydrogen-bond angles are rather greater than is normally considered permissible, although this may be consistent with the abnormally high NH-stretching frequency. The optical transform of this model, however, is in good general agreement with the observed diffraction data; while not correct in every detail, this strongly suggests that the general form of the backbone configuration is correct.

In conclusion, it seems to us highly probable that the collagen structure consists of three chains of the poly-L-proline type modified to give $3\frac{1}{3}$ residues per turn. These three chains are bound together by a system of NH . . . O hydrogen bonds between residues approximately on the same axial level, and the structure is further stabilized and the $3\frac{1}{3}$ residues per turn maintained by additional hydrogen bonding. It would seem that X-ray methods alone may not provide sufficient data to distinguish between different structures of this general type, and it is possible that the actual structure might be a complex of slightly different arrangements resulting in the maximum possible stability.

We are grateful to Prof. J. T. Randall for his continued advice and encouragement throughout the course of this research. Two of us (P. M. C. and S. McG.) are indebted to the Nuffield Foundation for research fellowships during the tenure of which this work was carried out. [Nov. 9

Note added in proof. Since this paper was submitted for publication a communication has appeared by Rich and Crick[24], in which they propose two alternative structures for collagen of the kind discussed above. They have called these collagen I and II. Each has a single system of NH . . . O hydrogen bonds, respectively in the anti-clockwise and clockwise arrangements.

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QUANTITATIVE SEPARATION OF B VITAMINS BY ELECTROPHORESIS ON AGAR PLATES

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We have already described the determination of components of a mixture of vitamin derivatives after chromatographic separation on circular paper[1]. This method makes a fairly exact estimation possible, but is not sufficiently sensitive for direct use with some biological material, owing to the limited quantity of material that can be placed on the paper. The lower limit of sensitivity of 0·05 μgm. for thiamine and 0·15 μgm. for nicotinic acid and derivatives requires a certain minimum concentration of these substances. The assay is, moreover, complicated by the difficulty of estimating microbiologically active derivatives not clearly separable by chromatography. Siliprandi and his collaborators[2] have applied electrophoresis on paper to the separation of derivatives of thiamine, riboflavin, pyridoxin and nicotinic acid which they later measured chemically. The sensitivity of their procedure (approximately 10 μgm.) can be greatly increased if electrophoresis is combined with a microbiological assay by applying the dried paper to a suitable medium. It is nevertheless limited by the fact that only about 10 mm.[3] of the solution can be applied to the paper.

We have developed a more suitable method by carrying out electrophoresis directly in thin layers of agar jelly as used in macro-electrophoresis[3]. Washed

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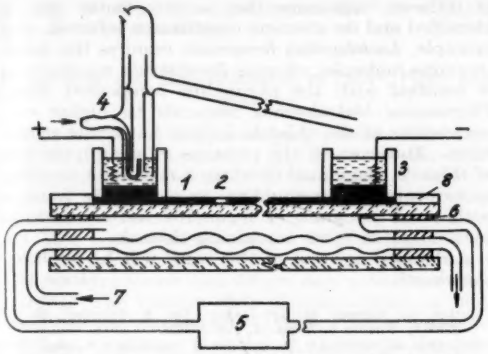

Fig. 1. Section of the apparatus for agar electrophoresis
1, agar (black); 2, starting hole; 3, electrode vessel; 4, pump; 5, thermostat; 6, rubber gasket; 7, cooling device; 8, glass frame

films of silica gel or polyvinyl alcohol have been used; possibly other transparent jellies could be used, but agar is to be preferred as a medium as it can be easily handled. High-voltage electrophoresis (up to 10,000 V.) is carried out in silicon films. After separation in the electric field, another layer of agar jelly is placed on the first. This second layer constitutes a medium containing all the substances required by the test organisms seeded into the agar except that to be determined. Zones of growth appear in those spots corresponding to the presence of the vitamin or its active derivative in the lower layer of the agar jelly. As in all diffusion tests, the logarithm of the dose is directly proportional to the diameter of the growth zone.

The vitamins can also be demonstrated by chemical reaction in the agar in the same way as on paper (see middle part of Fig. 2). Quantitative chemical determination is possible after cutting out and liquefying the appropriate portions of the agar jelly. For the separation of the thiamine group (shown in Fig. 2) the lower layer of agar should have a *p*H of 5·3. The warm, fluid agar is therefore diluted with phosphate buffer to a final concentration of 0·05 *M*. The most suitable concentration of agar must be determined for each system, and in general varies between 1 and 2 per cent. We use glass plates up to 60 cm. × 80 cm. framed by glass strips (2 cm. in breadth) which are caulked with agar to hold the liquid agar film on the previously warmed plate (Fig. 1, 8). Without appreciable warming of the agar film, 1–3 mm. thick, a current of 100–150 m.amp. can be put through it at a potential drop of 7 V./cm. Distilled water can be sprayed on the film should it dry up with prolonged use. Under these conditions the separation of thiamine and its derivatives is achieved in about four hours and can be controlled by the use of visible indicators such as acid fuchsin, methyl green or riboflavin. The test substances are placed in holes, 5–10 mm. in diameter, punched in the jelly with a cork borer along a starting line at intervals of 2–4 cm. (Fig. 1, 2). If necessary, another layer of agar may be added along the starting line to increase the thickness. In this way 0·2 ml. or more of substrate can be placed on the agar, the grading of the doses being preferably logarithmic. Glass cups or filter-paper disks can be used instead of the punched holes for accommodating the substrate. Even solid substances such as minced organs, fæces and also blood can be placed directly on the agar, after denaturation of protein, without previous extraction.

In certain instances, increased separation of the active growth factors can be achieved by a combination of paper chromatography and electrophoresis in agar jelly, strips of the chromatogram being placed either singly or packed along the starting line. A frame 3 cm. in diameter composed of glass strips which are fixed by clamps is set on the solid agar plate and filled with liquid agar to a height of about 5 mm. and serves as an electrode vessel (Fig. 1, 3). With narrow electrophoretic plates up to 10 cm. wide, two sintered glass filters (Eintauchnutsche Schott *G*4) can be used for the purpose with two carbon rods inserted to serve as electrodes. If the pores in the sintered glass filters are too big, they can be covered with agar. The apparatus includes a heating and cooling device and a pumping system for the electrolyte solution. Before the agar is poured on the glass plate, the plate is warmed by pumping water at 55° C. into a chamber formed by two glass plates and a soft rubber gasket (Fig. 1, 6). For cooling, cold water is passed through the chamber (Fig. 1, 7). Incubation of the test organisms is carried out overnight after the requisite adjustment of temperature in the thermostat (Wobser Ultrathermostat) (Fig. 1, 5). During long runs, the electrolyte solution is repeatedly transferred by a blower from the anode to the cathode vessel and vice versa (Fig. 1, 4) to keep the hydrogen-ion concentration around the electrodes reasonably constant.

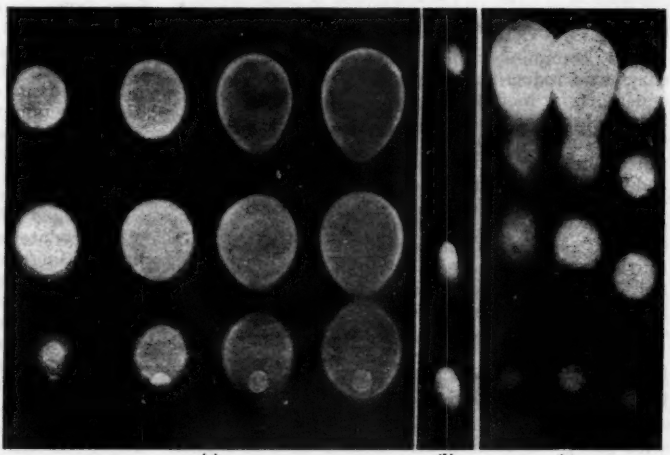

Fig. 2

(*a*) Growth zones with *Lactobacillus fermentum*. Standard mixture, 0·008–0·064 μgm. Bottom row, pyrophosphoric acid ester of thiamine; middle row, monophosphoric acid ester of thiamine. Top row, thiamine itself

(*b*) Chemical development to thiochrome (in ultra-violet light)

(*c*) Left: urine after a loading-test with thiamine. Centre: the same urine, but with added standard mixture of thiamine pyro- and mono-phosphoric acid ester, thiamine disulphide and thiamine. Right: the standard mixture alone

The growth zones shown in Fig. 2 were obtained with *Lactobacillus fermentum* **ATCC 9338**, by means of which as little as 0·002 μgm. of thiamine can be demonstrated. The diameter of growth zones is directly proportional to the logarithm of the dose within the limits 0·008–0·512 μgm. of thiamine (see Fig. 2). The pyrophosphoric acid and the monophosphoric acid esters of thiamine, thiamine itself and its disulphides and disulphide phosphates migrate to the cathode. For this reason the starting point for the thiamine group is placed near the anode. The speed of migration is in certain circumstances influenced by the ions present in the test solution. If necessary, the growth zones are identified by the use of an internal standard (see Fig. 2). We were able to demonstrate by such means the presence of thiamine disulphide in all the organs and excreta examined.

It is preferable to use for these tests organisms responding to as many factors as possible rather than the organisms of high specificity used for microbiological tests. It is by the different requirements of different organisms that a new factor can be identified and its chemical constitution inferred. For example, *Lactobacillus fermentum* requires the intact thiamine molecule, whereas *Rhodotorula mucilaginosa* is satisfied with the pyrimidine component alone. *Phycomyces blakesleeanus* responds to higher concentrations of the thiazole moiety but not to pyrimidine. However, in the presence of small quantities of thiazole, the mould develops a mycelium on those spots containing pyrimidine, or conversely forms it with small amounts (4 mgm./100 ml. medium) of pyrimidine on spots containing thiazole.

A detailed account of this work will be published elsewhere[4]. [June 14

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VALENCY CHANGES OF MOLYBDENUM DURING THE ENZYMATIC REDUCTION OF NITRATE IN *NEUROSPORA*

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MOLYBDENUM has been identified as a constituent of nitrate reductase in *Neurospora crassa*[1,2], *E. coli*[3] and in higher plants[4]. The micronutrient undergoes oxidation-reduction changes during nitrate reductase action in the following sequence:

Reduced triphospho-pyridine nucleotide → flavine adenine dinucleotide or flavine mononucleotide → molybdenum → nitrate ion (ref. 5)

This communication reports that, during the enzymatic reduction of nitrate, the valence change undergone by molybdenum involves Mo^{6+} and Mo^{5+}. Paper chromatography was used to prepare pure solutions of salts with molybdenum in various valency states, and for identifying Mo^{6+}, Mo^{5+} and Mo^{3+}. Sodium molybdate reduced with sodium dithionite, which acts as an electron donor for the enzymatic reduction of nitrate, was shown to contain Mo^{5+} as the most reduced valence state of the micronutrient. Further, Mo^{5+} prepared free from molybdenum in the other valence states acts as an effective donor for nitrate reductase action, but Mo^{6+} is without effect. Mo^{3+} will reduce nitrate non-enzymatically.

Determination of Valence States of Molybdenum present in Dithionite-reduced Sodium Molybdate

Mo^{6+} and Mo^{5+}. The molybdate solution reduced with sodium dithionite was spotted on acid-washed Whatman No. 1 paper, and run in the following solvent mixture: water 100, glacial acetic acid 10, potassium thiocyanate 10 gm. The chromatograms were dried and developed in one of three ways: (*a*) spraying with 0·2 per cent dithiol in 2 *N* hydrochloric acid; (*b*) spraying with a mixture of 10 per cent stannous chloride and 10 per cent potassium thiocyanate; (*c*) exposure to hydrogen chloride vapour.

R_F values (which had been checked with standard solutions of molybdenum of the two valencies) are: Mo^{6+} 0·3–0·4; Mo^{5+} 0·8.

An alternative method is the addition of 8-hydroxyquinoline in 50 per cent chloroform–ethanol to the dithionite-reduced molybdate solution. The chloroform layer containing the oxinates was spotted on Whatman No. 1 paper, dried and run in ethyl alcohol. The oxinate spots of Mo^{6+} and Mo^{5+} were observed in daylight or in ultra-violet light:

Mo^{6+} oxinate (yellow), $R_F = 0$, maxima 250, 370 mμ; minimum 280 mμ.

Mo^{5+} oxinate (reddish purple), R_F 0·9, maxima 243, 540 mμ; minimum 480 mμ.

These values had been determined using standard solutions of the molybdenum of the two valencies.

Mo^{5+} and Mo^{3+}. The dithionite-reduced solution of molybdate in hydrochloric acid was separated on paper using an ether–thiocyanic acid solvent prepared by adding 10*N* hydrochloric acid (40 ml.) to a solution of potassium thiocyanate (40 gm. in 40 ml. water) (the mixture was cooled to 15° and extracted with 150 ml. redistilled ether; the ethereal layer was filtered, and stored in a refrigerator). After drying, the paper was sprayed with 10-vol. hydrogen peroxide, then dried and sprayed again with a mixture of 10 per cent potassium thiocyanate and 10 per cent stannous chloride. No molybdenum valency less than 5 was detected in the reduced molybdate solution. Using standard solutions of Mo^{5+} and Mo^{3+}, R_F values in this solvent are 0·8 and 0·0, respectively.

Mo^{3+} is readily oxidized to Mo^{5+} in the presence of Mo^{6+}, so that the separation of Mo^{3+} in the presence of Mo^{6+} was not feasible. It has not been

possible to prepare and determine Mo^{4+} in solution because of its instability.

Preparation and Determination of Mo^{6+}, Mo^{5+} and Mo^{3+}

Mo^{6+} was prepared by acidifying sodium molybdate with 2*N* hydrochloric acid, and Mo^{5+} by dissolved molybdenum pentachloride in acetone. A chloride solution of Mo^{3+} was made by reducing a mixed solution of sodium molybdate (0·5 gm./5 ml. water) and 10*N* hydrochloric acid (10 ml.) with zinc dust until the solution ceased to give a red colour when aliquots were tested with 10 per cent potassium thiocyanate. The valency state of molybdenum in such a mixture was checked by reducing 1 ml. of a sodium molybdate solution containing 12·5 mgm. molybdenum per ml. as described. When all the zinc had dissolved, the solution was diluted with water to 100 ml.; 40–50 ml. aliquots were mixed with 50 ml. 2*N* sulphuric acid and titrated with standard potassium permanganate.

Exp. 1. 5,625 μgm. of reduced molybdenum required 3·5 ml. 0·05*N* potassium permanganate. Calculated for $Mo^{3+} \rightarrow Mo^{6+} + 3e$, 3·51 ml.; for $Mo^{4+} \rightarrow Mo^{6+} + 2e$, 2·34 ml.

Exp. 2. 5,000 μgm. of reduced molybdenum required 15·6 ml. 0·01*N* potassium permanganate. Calculated for $Mo^{3+} \rightarrow Mo^{6+} + 3e$, 15·62 ml.; for $Mo^{4+} \rightarrow Mo^{6+} + 2e$, 10·4 ml.

The zinc and traces of Mo^{5+} were removed from the solution as follows: 5 ml. of the mixture was placed on a cellulose column (15 cm. × 1·5 cm. internal diameter). When the solution had soaked in, the inner glass wall above the cellulose was wiped with filter paper to remove any adhering liquid. The column was then eluted with thiocyanic acid in ether, and this was continued for at least 15 min. after the red band of Mo^{5+} CNS had passed through the column. Ether was then passed through the column to remove thiocyanic acid, followed by distilled water to elute Mo^{3+}. The final eluate was warmed to 45° and carbon dioxide passed through to expel ether. The Mo^{3+} solution was diluted to 100 ml. with water and stored in the refrigerator.

The molybdenum concentrations in the solutions were determined as follows:

Mo^{6+}. To 5 ml. of sodium molybdate solution containing 2·5 mgm. molybdenum were added 5 gm. potassium thiocyanate and 10 ml. 5*N* hydrochloric acid; the mixture was kept at 50° for 15 min. and was diluted to 100 ml. with 10 per cent potassium thiocyanate in 2*N* hydrochloric acid. The solution was filtered through a No. 42 Whatman paper, and 10–20 ml. aliquots were extracted with 40–50 ml. redistilled ethyl acetate. The ethyl acetate extracts were measured at 490 mμ on a Spekker absorptiometer (blue-green filters). The concentration range used was 1–12 μgm. molybdenum per ml. ethyl acetate.

Mo^{5+}. To 1 ml. of molybdenum pentachloride in acetone containing approximately 500 μgm. molybdenum per ml. was added 10 ml. of 10 per cent potassium thiocyanate in 2*N* hydrochloric acid and the solution kept at 50° for 15 min. to reduce any Mo^{6+} to Mo^{5+}. Aliquots were extracted with 40–50 ml. ethyl acetate and determined as described previously.

Mo^{3+}. To 0·5 ml. of Mo^{3+} solution containing approximately 500 μgm. molybdenum per ml. was added 10 ml. of 10 per cent potassium thiocyanate in 2*N* hydrochloric acid followed by 10-vol. hydrogen peroxide until the red colour produced started to fade. Then 10 per cent stannous chloride in 2*N* hydrochloric acid was added until the restored red colour no longer deepened. The mixture was then shaken with 40–50 ml. of ethyl acetate; the ester layer was filtered, and the colour measured at 490 mμ.

Table 1. Mo^{3+} AS AN ELECTRON DONOR FOR THE ENZYMATIC REDUCTION OF NITRATE

Millimicromoles of nitrite formed in the reaction mixture

Exp.	Reduced triphos. pyr. nucleotide Enzyme Flavin mono-nucleotide Pot. nitrate	Mo^{3+} Enzyme Pot. nitrate	Mo^{5+} Pot. nitrate	Mo^{5+} Boiled enzyme Pot. nitrate	Mo^{6+} Enzyme Pot. nitrate
1	35·5	36	0	2	0
2	24	22	0	1	0

0·5 ml. of enzyme containing approximately 400 μgm. of protein, 0·20 ml. reduced triphosphopyridine nucleotide (2 μmol./ml.), 0·20 ml. flavin mononucleotide (0·09 μmol. per ml.), 0·6 ml. 0·1 *M* potassium nitrate and 50 μgm. Mo^{6+} or Mo^{5+} contained in 0·01 ml., each checked for purity by paper chromatography. The reaction mixtures were made to 2 ml. with 0·1 *M* phosphate buffer *p*H 7·5. After 10 min. at room temperature the mixtures were assayed for nitrite using sulphanilamide and *N*-(1-naphthyl) ethylenediamine reagents.

Enzymatic Reduction of Nitrate by Nitrate Reductase

Nitrate reductase was prepared from *Neurospora crassa* (Wild type 146, microconidial; kindly supplied by Dr. William D. McElroy, Johns Hopkins University, Baltimore) as described elsewhere[5]. Mo^{6+} and Mo^{5+} solutions, the valency purity of which was previously checked by paper chromatography, were tried as electron donors in the presence of the purified enzyme. The results are given in Table 1.

The results in Table 1 show that Mo^{5+} is as effective as reduced triphosphopyridine nucleotide as an electron donor for the enzymatic reduction of nitrate. Mo^{6+}, however, is without effect. In the absence of nitrate reductase or in the presence of the boiled enzyme, Mo^{5+} does not reduce nitrate. The very small amount of nitrite formed with the boiled enzyme is attributed to a release of bound nitrite from the denatured protein.

Non-enzymatic Reduction of Nitrate by Mo^{3+}

Mo^{3+} reduces nitrate non-enzymatically, whereas Mo^{5+} or Mo^{6+} is without effect. Thus it is possible to determine Mo^{3+} in the presence of Mo^{6+} by measuring reduction of nitrate. The rate of chemical reduction of nitrate by Mo^{3+} is approximately twice that of nitrate reductase action. Over the range 0·1–20 mμ *M* nitrate, 2 mμ *M* Mo^{3+} will reduce 1 mμ *M* nitrate chemically.

It is unlikely that the chemical reduction of nitrate by Mo^{3+} is important for the reduction of nitrate in *Neurospora*, because Mo^{3+} has not been detected in the felts and the enzymatic reduction involves Mo^{6+} and Mo^{5+} only.

It has not been possible to effect a non-enzymatic reduction of either Mo^{6+} to Mo^{5+}, or Mo^{5+} to Mo^{3+}, using reduced di- or tri-phosphopyridine nucleotide as an electron donor. Reduced triphosphopyridine nucleotide does not appear to reduce Mo^{5+} to Mo^{3+} in the presence of nitrate reductase. [July 8

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LETTERS TO THE EDITORS

The Editors do not hold themselves responsible for opinions expressed by their correspondents. No notice is taken of anonymous communications.

Function of Krause's Membrane

A. V. Hill[1] has shown that ordinary diffusion of a chemical substance is too slow to explain the rapidity with which the contraction in the interior of a striated muscle fibre is set off by the action potential, which is a change in the potential difference across the surface membrane; some more rapid link must therefore exist. Krause's membrane (*Z* line, telophragma) is a possible anatomical basis for such a link[2], since it extends continuously across the fibre and is attached to the interior of the sarcolemma[3,4].

We have tested this possibility by depolarizing very small areas of the surface of fresh fibres isolated from frog muscles. A micropipette with a squared-off tip, diameter about 2 microns, filled with isotonic sodium chloride, was firmly applied to the fibre surface and fed with pulses of current of $\frac{1}{2}$-sec. duration, making the interior of the tip about 20–40 mV. negative with respect to the Ringer bath. Fig. 1 shows that, when this was done opposite an *A* band, there was no contraction, while if the pipette was opposite an *I* band (with *Z* at its centre), that *I* band alone shortened, the contraction spreading only a short way in. In neither case was it possible to initiate shortening with pulses which hyperpolarized the membrane. Greater inward spread was obtained with a 4-micron pipette (Fig. 2), which is the largest that could be used without overlapping more than one *Z* line. The *I* band to which this pipette was applied now shortened for a distance of about 10 microns inward from the surface, while neighbouring *I* bands (1·5–3 microns from the central *Z* line) did not shorten and were, in fact, passively stretched.

The fibres had ceased to give propagated electrical activity by the time we made these observations, but were still capable of powerful local twitches in response to depolarization of a larger area of membrane. The current applied with the micropipette would not be expected to set up a propagated action potential even in an excitable fibre, because of the smallness of the area depolarized.

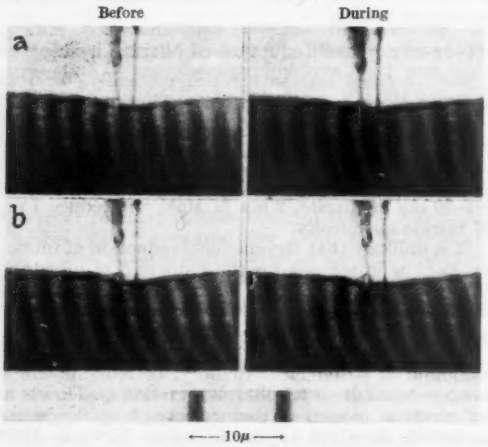

Fig. 1. Micropipette applied to edge of an isolated fibre from the semitendinosus muscle of the frog. Polarized light, compensated so that *A* bands appear dark. In (*a*), pipette is applied opposite an *A* band, in (*b*) opposite an *I* band. Left, before, and right, during, a pulse of current depolarizing the membrane opposite the tip. Contraction in (*b*) only, and then affecting only one sarcomere. In other experiments which were not recorded photographically, the contraction spread about 4 microns inwards

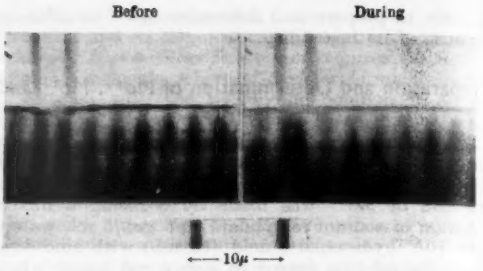

Fig. 2. As in Fig. 1,*b*, but with larger pipette. Contraction spreads about 10 microns inwards, but there is no spread to the adjacent sarcomeres, where the *I* bands are, in fact, stretched

We consider that these results are strong evidence that the influence of membrane depolarization is conveyed to the interior of the fibre by spread along some structure in the *I* band; from the anatomical situation, this must almost certainly be Krause's membrane.

This work was done during the tenure of a fellowship by one of us (R. E. T.) from the National Institute of Neurological Diseases and Blindness, U.S. Public Health Service.

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Sept. 14.

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Reaction of Starfish Spermatozoa to Histidine and Certain Other Substances considered in Relation to Zinc

Starfish spermatozoa are generally immobile in sea-water suspension. However, Metz[1] has reported that they become intensely active when they are suspended in egg albumin – sea water. His experiments were chiefly concerned with the agglutination reaction, and further it was shown (Metz and Donovan[2]) that most of the amino-acids and peptides were also capable of agglutinating the sperm, when fertilizin was added. Apart from the problem of agglutination, we were interested in the induced motility of these spermatozoa in albumin – sea water, and have examined the effects of various amino-acids on their motility and metabolism.

It was found that L-histidine exerts a marked effect on the motility of these spermatozoa. It is very striking to take a small amount (0·5 ml.) of the diluted sperm (1 : 20) of the starfish, which are almost motionless, and observe instantly active movements on addition of 0·5 ml. of histidine – sea water (2×10^{-3} *M*, *p*H 8·0). At the same time the effect of histidine on the initial oxygen uptake of these spermatozoa was examined by the usual Warburg technique, at 20° C. and with air in the gas phase. As shown in Fig. 1, histidine sharply increases the oxygen uptake, the effect being progressively increased when sea water containing 10^{-4}, 10^{-3} or

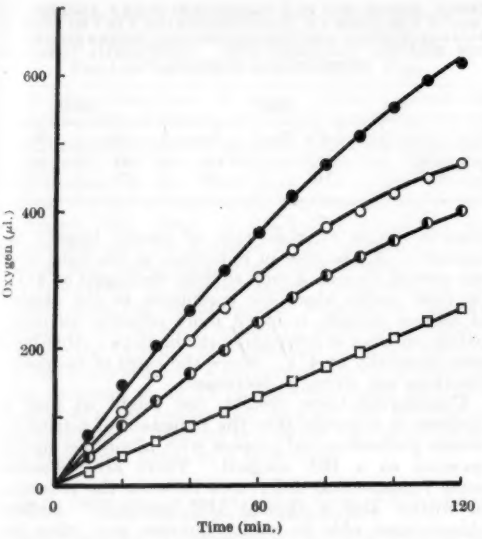

Fig. 1. Effect of histidine on the oxygen uptake of starfish spermatozoa in sea water with different concentrations of histidine: □—□—, control; ◐—◐—, 10^{-4}; ○—○—, 10^{-3}; ●—●—, 10^{-2} *M* histidine – sea water

10^{-2} *M* histidine is added to sperm suspensions. The motility of spermatozoa is also greatest in 10^{-2} *M* histidine – sea water.

Further experiments showed that cysteine and glycine are also effective, the motility and the rate of the respiration of the spermatozoa in cysteine – sea water being about the same as in histidine – sea water. The effect of glycine was very small. Also the chelating agents ethylenediaminetetraacetic acid and diethyldithiocarbamate proved to be effective, but the spermatozoa swim less actively in sea water containing these agents than in histidine – sea water. However, it is to be noted that no such effects were produced when sea water containing other amino-acids (alanine, leucine, serine, glutamic acid, arginine, methionine, cystine, tryptophan, tyrosine) was added to sperm suspensions.

The question immediately arises whether the extraordinary effect of histidine is essentially brought about by the liberation of zinc from the sperm, since there is evidence that the imidazole group of histidine has a strong zinc-binding capacity (Gurd and Goodman[3]) and since it has been shown (Fujii[4]) that zinc is present in considerable amount in some marine sperm, especially in the middle-piece and acrosome. That zinc occurs in high concentration in human spermatozoa has also been reported by Mawson and Fischer[5]. Therefore, the zinc content of *Asterias* spermatozoa was determined polarographically and a comparison was made between (1) sperm suspended in sea water for 30 min. and (2) sperm suspended for 30 min. in sea water containing 10^{-2} *M* histidine. It was found that the zinc content of the spermatozoa was greatly reduced after the treatment with histidine, suggesting that zinc or some zinc compound had passed from the sperm to the external solution. On the average, the zinc content of the former (1) was 0·21 μgm./mgm. dry weight, while that of the latter (2) was 0·10 μgm./mgm. dry weight. The external medium, which had contained histidine-treated sperm, was found to give a positive dithizone reaction after centrifugation.

The evidence so far obtained suggests that, at least in the starfish, the release of zinc in minute quantities from the sperm is responsible for the initiation of the sperm's activity, both as regards motility and respiration. It is likely that the sperm middle-piece or the acrosome loses zinc to histidine or other metal-chelating substances, and that the liberation of zinc itself represents the sudden release of a state of stress in the spermatozoa. It appears difficult for the present to discuss these results in relation to the dilution effect (Rothschild and Tyler[6]) or the anaerobic motility induced by glycine (Tyler and Rothschild[7]) in sea-urchin sperm. It is interesting, however, to note that the effect of histidine was scarcely observable in a very dilute suspension of the starfish sperm. Could zinc release be normally involved in the basic mechanism of the motility of the spermatozoa ?

These experiments described form part of a wider investigation on the physiological role of zinc. One of us (Fujii[8]) has already indicated that zinc is present in appreciable amount in the nucleoli of starfish oocytes, and is possibly involved in the mitotic processes.

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Role of Photochemical Processes in the Reduction of Nitrate by Green Algae

Experiments have been described supporting the idea that reduction of nitrate in the light by green algae is a photochemical process[1]. However, a strong argument against this assumption is the fact that, without carbon dioxide, a really significant reduction of nitrate in the light has never been found[2,3]; only several hours after the addition of glucose can a strong evolution of oxygen due to nitrate reduction be observed[3]. This indicates an indirect influence of light via the formation of photosynthetic products rather than a direct photochemical reduction of nitrate. In the following we show that it is necessary to distinguish between the effect of light on the reduction of nitrate and nitrite, respectively (cf. also Harvey's results with diatoms[4]). The latter is the first intermediate in the reduction of nitrate by green algae[5].

When nitrate is added to *Ankistrodesmus braunii* suspended in nitrogen-free culture medium in the absence of carbon dioxide, only a minute amount of oxygen is evolved (Fig. 1). This result, obtained manometrically under a variety of experimental conditions (*p*H 6·5 and 4·5, under pure nitrogen and air, with 4×10^{-4} to 9×10^{-3} *M* potassium nitrate,

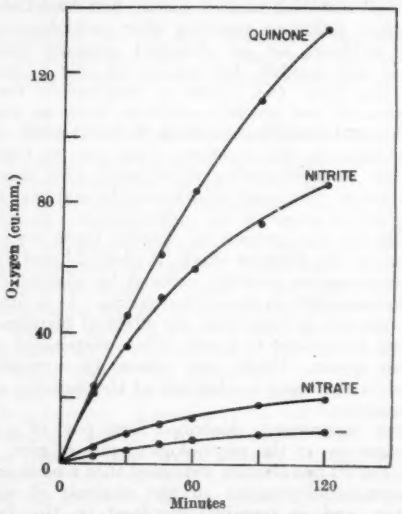

Fig. 1. Oxygen evolution after addition of quinone, sodium nitrite, and potassium nitrate (no addition as control) to *Ankistrodesmus* suspended in nitrogen-free culture medium (*p*H 6·5) in the absence of carbon dioxide. Gas phase, nitrogen (potassium hydroxide in the centre well); light intensity, 1,300-foot candles; temperature, 15° C.; 21 mm.³ cells (6·5 mgm. dry weight) per vessel. Concentrations of oxidants: quinone, $3 \cdot 3 \times 10^{-3}$ *M*; sodium nitrite, $1 \cdot 1 \times 10^{-3}$ *M*; potassium nitrate, $8 \cdot 3 \times 10^{-4}$ *M*. Theoretical yield: 179 mm.³ oxygen in each case. Pretreatment of the algae: 4 hr. in nitrogen-free medium at moderate light intensity (400 ft.-candles); gas phase, air

at 15° and 25° C.), is in excellent agreement with earlier findings[2,3]. The rate of reduction of nitrate under these conditions is approximately the same as that normally occurring in the dark. On the other hand, reduction of nitrate in the presence of carbon dioxide, or in the absence of carbon dioxide with added glucose[3], proceeds about ten times as fast as reduction in the dark[4]. By contrast, an addition of nitrite in non-toxic concentrations produces an immediate strong evolution of oxygen in the light. (Pressure changes are negligible in the presence of alkaline pyrogallol as an oxygen-absorbing agent.) Per mole of nitrite reduced, 1·5 moles of oxygen are formed. The rate of this reaction at *p*H 6·5 is somewhat smaller than that of the quinone–Hill reaction under the same conditions (Fig. 1). But at *p*H 4·5 reduction of nitrite in the light proceeds much faster, the rate of the reaction being nearly identical with that of the quinone–Hill reaction at *p*H 6·5. It is of interest in this connexion to note that the influence of *p*H on the dark-nitrite reduction is also strong but in the opposite direction[5]. Furthermore, it should be mentioned that an addition of nitrite in the dark under aerobic conditions leads to a strong increase in respiratory oxygen uptake[5], whereas under anaerobic conditions no gas exchange occurs.

In order to inhibit enzymatic processes effectively without influencing photochemical reactions possibly involved in the reduction of nitrite, the rate of the reaction under different conditions was studied at low temperatures. Comparison of the results obtained in a series of experiments performed at 4° C. and at 15° C., respectively (Table 1), shows that: (1) the reduction of nitrite at 15° C. in the light proceeds about twice as fast with concomitant photosynthesis as it does in the absence of carbon dioxide; (2) at 4° C. the favourable influence of carbon dioxide and

Table 1. Nitrite ($M \times 10^{-4}$) Reduction by 25 mm.³ *Ankistrodesmus* Cells in 1 hr. at *p*H 4·5. Sodium Nitrite ($5 \cdot 5 \times 10^{-4}$ *M*) added to Algae in Nitrogen-free Culture Medium. Nitrite Concentration measured Colorimetrically. Experimental Conditions otherwise as indicated in Fig. 1

	Light		Dark	
	$N_2 + 4\%\ CO_2$	N_2	Air	N_2
15° C.	212	108	40	20
4° C.	110	90	20	8

photosynthesis on reduction of nitrite largely disappears; (3) the rate of reduction in the light without carbon dioxide is only slightly decreased at 4° C.; (4) light under anaerobic conditions in the absence of carbon dioxide is much more effective in nitrite reduction than is respiration in the dark; this holds true especially at 4° C., where the rates of enzymatic reactions are strongly decreased.

Considering these results, one might at first be inclined to conclude that the reduction of nitrite is a simple photochemical process with nitrite acting like quinone as a Hill reagent. There are, however, some fundamental differences between the reduction of nitrite and a typical Hill reaction. Isolated chloroplasts, able to reduce quinone and other Hill reagents, are unable to reduce either nitrate[6] (unless nitrate reductase and triphosphopyridine nucleotide are added[7]) or nitrite[5,7]. Furthermore, the rate of nitrite reduction in the absence of carbon dioxide by whole *Ankistrodesmus* cells decreases faster than that of quinone reduction (Fig. 1). Upon a second addition of quinone, oxygen evolution immediately starts again at a high rate; with nitrite, however, only a small increase in oxygen formation is observed. The rate of nitrite reduction is apparently determined by the supply of carbon compounds necessary for the further assimilation of the products of nitrate and nitrite reduction[8].

Accordingly, the influence of light upon nitrate reduction in green algae might be explained in the following way. The first step (nitrate → nitrite), which proceeds rapidly only in the presence of carbon dioxide or of glucose[3], seems to be indirectly accelerated by light via the formation of photosynthetic products. On the other hand, the reduction of nitrite, which goes on at a high rate even at very low temperatures and in the absence of carbon dioxide, seems to be more closely connected with photochemical processes.

A full account of these and other results will be published later. My thanks are due to the U.S. National Academy of Sciences and to Foreign Operations Administration for a research scholarship.

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Respiratory Increase and Phosphorus and Nitrogen Metabolism in Sweet Potato infected with Black Rot

In the black rot of sweet potato caused by *Ceratostomella fimbriata* we found a respiratory increase—a characteristic of plant tissues infected with pathogens. We have investigated the rate of respiratory increase in the sound part next to the infected tissues during a week after the inoculation of the pathogen, the effect of 2,4-dinitrophenol on the respiration and the changes in the amount of phosphorus and nitrogenous compounds present.

In contrast to the constant respiration in the control and the equal rate of respiratory increase (about 80–90 per cent) caused by addition of dinitrophenol, the increase of respiration in tissue affected by black rot reached a maximum (about 90–100 per cent increase) at 48–72 hr., and gradually decreased thereafter; the respiratory increase due to addition of dinitrophenol dropped to *c.* 30 per cent at 48–72 hr., but recovered again at a later stage.

Table 1. Respiration of the Black-Rot Tissue of Sweet Potato and the Effect of Dinitrophenol

Twenty slices (7 mm. in diameter and 0·5 mm. thick) were put into the Warburg vessels containing 85 μmoles of phosphate buffer (pH 5·5) and 162 μmoles of sucrose in a total volume of 2·0 ml. Gas phase, air; temperature, 30°; 20 min. equilibration; dinitrophenol was added in a final concentration of 5×10^{-4} *M*

(hr.)	0	24	48	72	168
Control (μl.)	77·3	88·0	80·5	77·6	93·7
Control + DNP	138·7	156·0	143·0	149·0	178·0
Increase (%)	80	77	78	92	90
Black rot (μl.)		132·7	147·0	177·0	120·0
Black rot + DNP		208·0	186·0	232·0	231·0
Increase (%)		56	26	31	93

Since the respiratory increase due to dinitrophenol is dependent on the acceleration of the reaction (ATP → ADP + P_i), its effect indicates the speed of the above reaction in tissues. It is supposed that, therefore, the decrease of the effect in one stage may be caused by the promotion of breakdown and/or utilization of adenosine triphosphate by some biological process. To clarify this point the tissues were analysed for inorganic phosphate and acid-soluble organic phosphate. Both phosphate fractions of the control remained almost constant, whereas inorganic phosphate in black rot tissue decreased at first, increased in the second stage, and then dropped again; a concomitant increase of acid-soluble organic phosphate was observed. Occasionally it was observed that the level of inorganic phosphate in the secondary stage was not raised but remained constant. There was no measurable change in the alcohol-soluble phosphate fraction containing phospholipid and the phosphate fraction extractable with cold perchloric acid and containing ribonucleic acid. The accumulation of acid-soluble organic phosphate may indicate the activation of a phosphate system by which the energy of adenosine triphosphate is utilized and the rate of generation of adenosine diphosphate is increased; thus the increased respiration in the black-rot tissue of sweet potato may be linked to the activated phosphate system.

To elucidate the anabolic process such as the activation of the phosphate cycle in the black-rot tissues, we have examined the nitrogenous metabolism. Compensating the consumption of the acid-soluble nitrogenous fraction (such as amino-acids), a concomitant increase of the acid-insoluble nitrogenous fraction (such as protein) was observed; in further experiments, the synthesis of functional protein such as mitochondrial protein was observed.

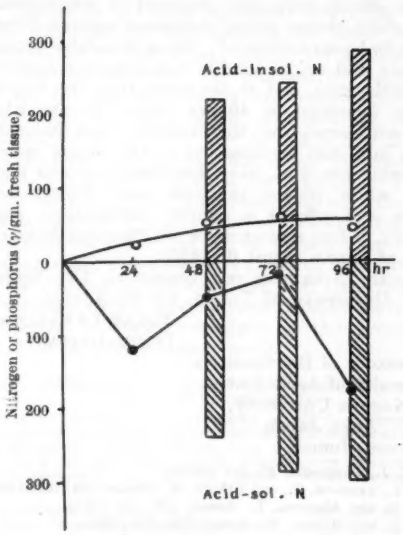

Fig. 1. Change of the amounts of phosphorus and nitrogenous fractions in the sweet potato infected with black rot. Inorganic phosphate, —●—, is expressed as the difference between the control and that of black-rot tissue; acid-soluble organic phosphate, ○—○, as the difference between that in black-rot tissue and that in the control. In the same way, acid-soluble nitrogen shows the decrease and acid-insoluble nitrogen the increase over the control. The values for the controls scarcely changed through the experimental stage

The mechanism of the respiratory increase in the black-rot tissues is thought to be as follows: by the penetration of *Ceratostomella fimbriata*, the protoplasm of the sound part next to the infected tissue is activated to synthesize organic phosphate, functional proteins such as mitochondria, and simultaneously accelerates the adenosine phosphate reaction (ATP → ADP + P_i); this results in an increase of respiration. P. J. Allen[1] believed that the respiratory increase in wheat infected by powdery mildew could be explained by the mildew toxins acting as uncouplers and accelerating the production of adenosine diphosphate. We have put forward the view that a part of the respiratory increase might be caused by the uncoupling effect of ipomeamaron, a sesquiterpene $C_{15}H_{22}O_2$, and related substances accumulated in the infected sweet potato tissues[2]; but from the above results it is thought that the effect of uncouplers such as ipomeamaron may be only partly responsible for the respiratory increase. As these uncouplers acted as inhibitors of the phosphate metabolism and protein synthesis of *Ceratostomella fimbriata*, they were presumed to take part in the resistance of sweet potato against the pathogen[3].

The metabolites (essential oils (such as ipomeamaron), polyphenols (for example, chlorogenic acid), coumarin substances (for example, umbelliferon and scopoletin)) were accumulated in the sound tissue, paralleling the respiratory increase[4], and offered resistance to infection by the pathogen. In addition, a thermolabile and non-dialysable fraction containing an inhibitor is produced in the black-rot tissues[5].

Those effects were also observed in white potato tissue, which shows strong resistance against *Ceratostomella fimbriata* infection[5]; the acid-soluble organic phosphate and acid-insoluble nitrogenous compound were synthesized, and at the same time the respiration was increased (c. 40 per cent). In the white potato rot-tissue, the thermolabile, non-dialysable fraction inhibited germination of the fungal spores.

K. Tomiyama *et al.* have described a similar effect in the white potato infected with *Phytophthora infestans* and offered a similar explanation, independently, before a meeting of the Phytopathological Society of Japan (April 6, 1955).

We wish to express our thanks to Dr. Yusuke Sumiki, University of Tokyo, for his advice.

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Molecular Weight of Insulin in N,N-Dimethylformamide

The detailed investigations of Sanger and co-workers have established the molecular weight of insulin as some multiple of about 6,000[1]. It is not clear, however, whether the fundamental covalently bonded unit is the 6,000 or 12,000 species. (The determination of the positions of the S—S linkages in insulin[1] does not establish the fundamental unit as the 6,000 species. The methods of analysis do not preclude the possibility of a staggered arrangement of the S—S linkages such that the 12,000 species is covalently bonded together.) Several workers using a variety of techniques—osmotic pressure, sedimentation and light-scattering—achieved results indicating that the minimum molecular weight of insulin in aqueous solution is 12,000[2]. However, other work has indicated a value of 6,000. Harfeneist and Craig[3], by counter-current distribution methods in 2-butanol-dichloracetic acid systems, Fredericq[4] by sedimentation-diffusion studies in alkaline solutions, and Kupke and Linderstrøm-Lang[5] by osmotic pressure determinations in aqueous guanidine chloride solutions, have all recently contributed data consistent with the value of 6,000. Osmotic pressure studies of insulin in N,N-dimethylformamide in this laboratory also indicate a molecular weight of 6,000 for the fundamental unit.

Amorphous insulin (lot 200-1*B*-11*J*) was a gift of the Eli Lilly Company. N,N-Dimethylformamide (DuPont) was purified by distillation at reduced pressure through an activated charcoal column. Although it has a relatively low dielectric constant of 26·6, the osmotic pressure studies were made in 0·2 *M* potassium iodide solutions of N,N-dimethylformamide in order to minimize any possible Gibbs–Donnan effect. Zimm–Myerson osmometers[6] were used with suitably permeable membranes prepared from Visking cellulose casing by a treatment which included an 18-hr. soak in 80 per cent ethanol. The osmometers were placed in solvent inside closed glass jars to prevent absorption of water. Equilibrium was attained in about two days and measurements were made for at least three days longer. The temperature was 13·8° C. and was regulated to ± 0·01° C.

Table 1

Insulin conc. (gm./l.)*	π (cm. H_2O)	$\bar{M}_n$ × 10^{-3}
0·36	1·29	6·7$_6$
0·46	1·64	6·8$_8$
0·88†	3·15	6·8$_4$
0·92	3·02	7·4$_4$
0·92	3·06	7·3$_4$
0·92	3·13	7·1$_4$
1·49	5·04	7·1$_4$
1·79	5·82	7·4$_7$
1·79	6·03	7·2$_8$

* In 0·2 *M* potassium iodide in N,N-dimethylformamide.
† In 0·1 *M* sodium nitrate in N,N-dimethylformamide.

The data from these experiments (Table 1 and Fig. 1) extrapolate to a molecular weight of 6,200 ± 300 at $C = 0$. Assuming that the deviation from ideality at higher concentrations is entirely due to a monomer–dimer equilibrium, $2I \rightleftharpoons I_2$, we calculate an association constant of 2×10^3 litre-mol.$^{-1}$, corresponding to $\Delta F^\circ = -4{\cdot}4$ kcal./mol.

The question arises as to whether the fundamental structure of insulin is disrupted in N,N-dimethylformamide. Facilities for an assay of the activity of insulin recovered from it were not available. However, the following test was applied. It is well known that insulin aggregates to a considerable extent in aqueous solution, and the extent of this aggregation, measured by the sedimentation-rate, is presumably a characteristic of the unaltered molecule. Sedimentation experiments were therefore performed on insulin, which, after solution in N,N-dimethylformamide, was dialysed against phosphate buffer, *p*H 7·5, Γ/2 0·1, in which it was completely soluble. Experiments were also performed with insulin dissolved directly in this buffer, for comparison. Within experimental error, there was no difference between the sedimentation constants, S^W_{20} (Table 2), for the original and the recovered insulin.

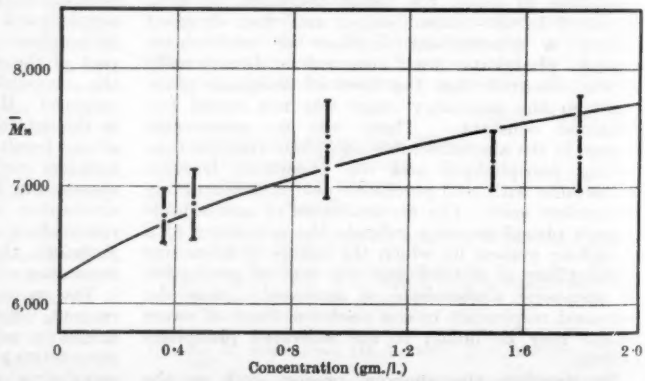

Fig. 1. Apparent number-average molecular weight of insulin in N,N-dimethylformamide as a function of concentration. The theoretical curve for a monomer–dimer equilibrium, with an association constant of 2×10^3 l./mol.$^{-1}$, is drawn through the data

Table 2

Sample of Insulin	C (gm./l.)	S^{w}_{20} (svedbergs)
Dissolved directly	5·1	3·3$_8$
	2·9	2·9$_8$
Recovered from N,N-dimethyl-formamide	5·1	3·1$_5$
	2·5	2·8$_4$

Although minimal molecular weights of 6,000 and 12,000 have been reported, there is no inconsistency necessarily involved. The value of 12,000 has been found in aqueous solutions, in which it is not unlikely that the dimer persists. Two reports of the value 6,000 have now been obtained independently by Harfeneist and Craig[3] and in this communication, in non-aqueous solvents, in which the aggregation reactions are apparently less favoured, and the true minimal molecular weight is observed.

This work is part of a study of the solubilities and properties of proteins in non-aqueous solvents, which will be reported in detail elsewhere.

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Inhibition of Complement Activity by Di-*isopropyl* Fluorophosphate

It is generally agreed that di-*iso*propyl fluorophosphate acts specifically to inhibit enzymes with esterase activity[1]. In the light of this conclusion, the finding that it inactivates guinea pig complement (C') is felt worthy of this brief communication. (In a personal communication, Dr. Laurence Levine reported that he has independently observed this same finding.)

When di-*iso*propyl fluorophosphate in concentrations of $8{\cdot}0 \times 10^{-3}$ to $1{\cdot}0 \times 10^{-4}$ M was incubated with diluted or undiluted guinea pig serum up to 4 hr. at 37° C., or overnight at 26° or 4°, and then the serum diluted and tested for complement activity, no effect of the fluorophosphate could be detected. When, however, $8{\cdot}0 \times 10^{-3}$ M di-*iso*propyl fluorophosphate was incubated for ½ hr. at 37° with varying concentrations of 1–100 diluted guinea pig serum in the presence of sheep cells sensitized with rabbit hæmolysin, 75–80 per cent inhibition of the hæmolytic activity of complement was noted. In the same circumstances, $3{\cdot}5 \times 10^{-3}$ M di-*iso*propyl fluorophosphate gave 10 per cent inhibition. On testing the dialysed supernatants from the latter reaction for the presence of the four components ($C'1$, $C'2$, $C'3$, $C'4$) of complement by the usual methods[2], only the first component ($C'1$) was found to have been appreciably inactivated.

That the effect of di-*iso*propyl fluorophosphate is on $C'1$ was confirmed by using sensitized sheep cells containing the first, second and fourth components (*Ea* $C'1$, 4, 2). These were prepared as described by Levine *et al.*[3] and incubated overnight at 20° with di-*iso*propyl fluorophosphate, then washed, resuspended in buffer and tested for activity with reagents specific for the respective components. As can be seen in Table 1, the concentrations required for inhibition of the $C'1$ activity of these cells varied from $2{\cdot}7 \times 10^{-3}$ to $8{\cdot}3 \times 10^{-5}$ M. This is considerably less than the molar concentrations ($8{\cdot}0 - 3{\cdot}5 \times 10^{-3}$) required for inhibition of C' for whole serum. The $C'4$ activity was not affected by this treatment. The effect of di-*iso*propyl fluorophosphate on $C'2$ could not be tested, since $C'2$ was spontaneously inactivated on standing in buffer alone. Partial reversal of the inhibition of $C'1$ activity could be obtained by allowing the cells treated with fluorophosphate to stand overnight in the presence of $0{\cdot}1$ M nicotinohydroxamic acid[4].

Table 1. Inhibition of $C'1$ on *Ea*C' 1, 4, 2, incubated with Di-*iso*propyl Fluorophosphate

$1{\cdot}0 \times 10^{9}$ cells/ml. were incubated at 30° C. overnight with saline–veronal–bicarbonate buffer to which was added the di-*iso*propyl fluorophosphate, $0{\cdot}001$ M calcium ions, $0{\cdot}003$ M magnesium ions, and $0{\cdot}04$ M imidazole buffer *p*H 7·6. The cells were then centrifuged, washed, resuspended in buffer and reacted with R_1, the reagent for testing the $C'1$ reaction (ref. 2).

Molar concentration of DFP ($\times 10^{4}$)	270	41	21	8·3	0
Time (min.) required to attain 50 per cent hæmolysis	>30	29	9	4	1

From this it is concluded that the first component of complement $C'1$ is an enzyme with esterase activity which exists in serum in a precursor form resistant to the action of di-*iso*propyl fluorophosphate. In the course of the immune hæmolysis reaction, the $C'1$ precursor is changed to an active form of $C'1$ which is now capable of being inhibited by the fluorophosphate. The $C'1$ on the 1, 2, 4 cells apparently exists in such an active form, or was possibly changed into such an active form, during incubation with the fluorophosphate.

I am grateful to Dr. C. T. Harwood and to Dr. Bernard Jandorff for supplies of di-*iso*propyl fluorophosphate, and to Dr. I. B. Wilson for a sample of nicotinohydroxamic acid.

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Occurrence of an L(+)-Alanine-Dehydrogenase in *Bacillus subtilis*

The metabolic analysis of mutant strains of *B. subtilis* has shown that the ability to use the ammonium ion as the sole source of nitrogen for growth corresponds to the presence of a glutamic-dehydrogenase[1]. However, a mutant unable to use the ammonium ion (strain S^{-}), lacking glutamic-dehydrogenase, oxidizes glutamic acid very actively. This strain may grow, with aeration, in a culture medium containing: $l(+)$-glutamate $0{\cdot}01$ M, ammonium sulphate $0{\cdot}01$ M, phosphate buffer (KH_2PO_4–Na_2HPO_4) $0{\cdot}02$ M, *p*H 7·5, sodium chloride $0{\cdot}01$ M, potassium sulphate $0{\cdot}01$ M, magnesium sulphate $0{\cdot}001$ M and ferric citrate $0{\cdot}0004$ M.

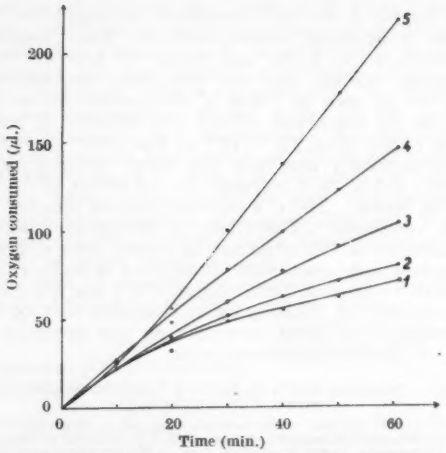

Fig. 1. Oxidations followed with a Warburg apparatus at 35° C. Each vessel contains: 2·0 ml. cell suspension (optical density 2·16) in 0·05 *M* phosphate buffer, *p*H 6·7; 0·2 ml. 5 *N* sodium hydroxide in the central cup. Curve 1, without substrate. Additions: curve 2, 100 µmole glycine; curve 3, 100 µmole *l*(+)-aspartate; curve 4, 100 µmole *l*(+)-alanine; curve 5, 100 µmole *l*(+)-glutamate

The absence of the glutamic-dehydrogenase in the S^- strain implies an oxidative pathway of glutamic acid different from the ordinary one.

α- and γ-Aminobutyric acids are slowly oxidized by this bacterium, and the test for glutamic-decarboxylase in extracts is negative. This finding suggests that an oxidation preceded by a decarboxylation is not operative in this case. Another possibility might be the oxidation of a compound formed by transamination of glutamic acid, according to reactions (1) and (2):

$$\text{Glutamic acid} + R\text{—CO—COOH} \rightleftharpoons R\text{—CH(NH}_2\text{)—COOH} + \text{α-ketoglutaric acid} \quad (1)$$

$$R\text{—CH(NH}_2\text{)—COOH} + O_2 \rightarrow R\text{—CO—COOH} + H_2O + NH_3 \quad (2)$$

$$\text{Glutamic acid} + O_2 \rightarrow \text{α-ketoglutaric acid} + H_2O + NH_3 \quad (3)$$

In this system, α-ketoglutaric acid is used as the source of carbon for growth. The α-keto-acid, which is the acceptor of $-NH_2$, is regenerated by oxidation. This mechanism implies that the α-keto-acid originates from cell reserves, and is not lost by any secondary reaction. These restrictive conditions are not necessary if the α-ketoglutaric acid can regenerate the α-keto-acid. If we suppose that the α-ketoglutaric acid is oxidized through the Krebs cycle, the *R*—CO—COOH compound could be oxalacetic acid or its decarboxylation product, pyruvic acid, or glyoxylic acid arising from the decomposition of citric acid[3]. In this case, *R*—CH(NH$_2$)—COOH would be aspartic acid or alanine or glycine. Of these three substances, alanine is readily oxidized, aspartic acid is less oxidized and glycine almost not at all (Fig. 1).

B. subtilis S^- cells, grown on the medium described before, harvested when in the exponential phase, and treated with lysozyme[3], give an extract which is still able to absorb oxygen. The utilization of oxygen is reinforced by addition of alanine and, to a lesser degree, by aspartic or glutamic acid. This oxygen consumption could result from the presence of a flavoproteic *l*-amino-acid-oxidase, such as in *Neurospora crassa*[4], which oxidizes *l*(+)-alanine, *l*(−)-phenylalanine and, less actively, glutamic acid and aspartic acid. However, an extract prepared from 12·5 mgm. lyophilized bacteria per ml., treated with lysozyme and centrifuged at 80,000 *g* for 45 min., gives a clear supernatant which does not oxidize *l*(+)-alanine with oxygen, but in which the alanine intensively reduces diphosphopyridine nucleotide (DPN+). The same extract, precipitated by ammonium sulphate at saturation, redissolved and dialysed against phosphate buffer (0·05 *M*, *p*H 7·3) gives the activity reported in Fig. 2.

l(+)-Glutamic acid, *l*(+)-aspartic acid, *l*(−)-phenylalanine and *l*(−)-leucine have no action. Triphosphopyridine nucleotide is not reduced in the same conditions.

As shown in Fig. 2, the maximum reduction of diphosphopyridine is a function of the concentration of alanine. An equilibrium is obtained, as is also evident from the re-oxidation brought about by addition of ammonium ions and to a lesser degree by hydrogen ions. The oxidation product of *l*(+)-alanine is pyruvate. By incubation, during 30 min. at room temperature and *p*H 8·75, of a mixture of 0·4 ml. of extract, 20 µ*M* of *l*(+)-alanine and 0·36 µ*M* of diphosphopyridine and 0·4 ml. of pyrophosphate buffer 0·1 *M* in a total volume of 1 ml., 0·31 µ*M* of pyruvate is formed. The pyruvate gives a reoxidation of the diphosphopyridine of the same magnitude as the ammonium ion.

One can conclude that the extract contains an enzyme which catalyses the reversible reaction (4).

$$CH_3\text{—CH(NH}_3\text{)}^+\text{—COO}^- + DPN^+ + H_2O \rightleftharpoons CH_3\text{—CO—COO}^- + DPNH + H^+ + NH_4^+ \quad (4)$$

This enzyme may be called '*l*(+)-alanine-dehydrogenase', in conformity with *l*(+)-glutamic-dehydro-

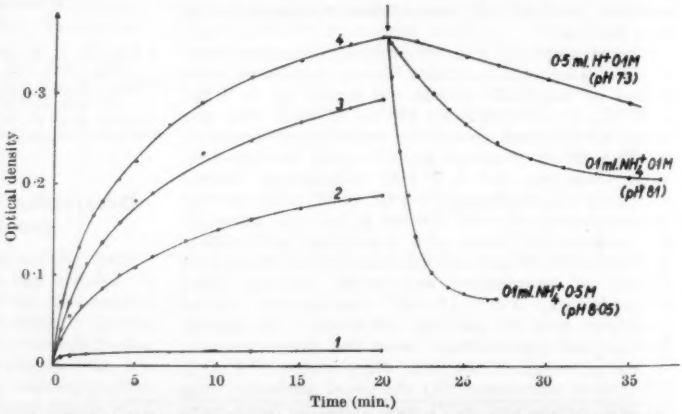

Fig. 2. *l*(+)-Alanine dehydrogenation followed by change in optical density of diphosphopyridine nucleotide at 340 mµ, with a Beckman Model *D.U.* spectrophotometer, at room temperature. The reaction mixture contained: 0·5 ml. cell-free extract, 0·5 ml. 0·1 *M* sodium pyrophosphate, 1·0 ml. 0·1 *M* phosphate buffer, *p*H 7·3; final *p*H, 8·1. Curves 1, 2, 3 and 4 correspond respectively to 0, 2, 5 and 10 µmole of *l*(+)-alanine in a volume of 0·5 ml. At zero time, 0·3 ml. of 0·001 *M* diphosphopyridine nucleotide is added. At the arrow, addition of ammonium ion and hydrogen ion as indicated on the curves. Values are corrected for dilution by the different additions

genase, the only comparable enzyme known. Like the former, this enzyme is very specific, since it does not act on *d*(−)-alanine and the other compounds tested.

The reversibility of reaction (4) shows that this enzyme is important, not only in the oxidation of *l*(+)-alanine, but also in the assimilation of ammonium. Although the assimilation of ammonium has only been shown to occur through glutamic-dehydrogenase[1], other observations suggest that different mechanisms may exist[5].

The S^- strain used in this work does not grow on alanine as sole nitrogen source, and in this case the amination of pyruvic acid by ammonia is not operative.

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Action of Ribonuclease on Living Cells *in vitro* and Synthesis of Deoxyribonucleic Acid

The action of ribonuclease on living cells from various materials has recently been studied[1,2]. Chick fibroblasts and myoblasts *in vitro* are readily affected; growth of the cultures and the number of mitoses are considerably reduced[2]; cytoplasmic and nucleolar basophilia is diminished or suppressed and a typical modification of the chromatin, seen both with phase-contrast optics in living cells and on fixed preparations, is observed. Many nuclei (up to 95 per cent when the action of ribonuclease has been particularly intense) contain granular, or granular and filamentous, material or even are prophasic in appearance[3]. Even when many nuclei are showing this prophasic aspect, few—if any—other mitotic stages are encountered.

Histospectrophotometric measurements were undertaken to assess how the hydrolysis of ribonucleic acid affected deoxyribonucleic acid metabolism. The equipment and techniques used for quantitative Feulgen determination after osmium fixation will be described in detail elsewhere. They are essentially similar to those of the Pollister and Leuchtenberger school. Measurements of randomly chosen intermitotic nuclei (all non-overlapping nuclei in a straight line tangential to original explants, several explants being used for each group) were measured at 546·1 mμ. The most striking results were obtained with cultures which had had a long contact (3 days) with ribonuclease (crystallized ribonuclease G.B.I.). In Fig. 1, the histogram for such cultures is compared to normal

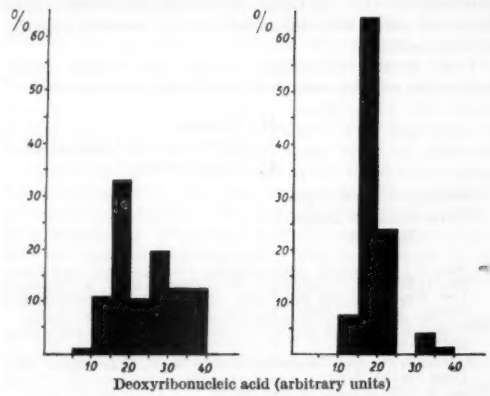

Fig. 1. Histograms of deoxyribonucleic acid values (arbitrary units) in intermitotic nuclei of 3-day old chick fibroblasts and myoblasts cultures. On the left, cultures treated with ribonuclease (1/300) in the medium (number of measurements, 108). On the right, controls of the same age (number of measurements, 81)

controls of the same age. There is a considerable decrease in the percentage of the cells with a normal diploid value (1·82 ± 0·17 in arbitrary units) and a considerable increase in the number of cells with a tetraploid (3·65) or intermediate value. Other cultures in contact with ribonuclease for a shorter time (15 hr.–2 days) show a similar but less marked shift in deoxyribonucleic acid values.

It should be emphasized that the granular distribution of deoxyribonucleic acid in many of the affected cells increases the heterogeneity of the dye distribution in the nucleus, and thus the measured extinction is lower than it would be if the same material was as homogeneously distributed as it is in the controls. A number of deoxyribonucleic acid values can be considered as too low, and the difference from the controls is certainly more marked than the histogram shows. In order to eliminate this lowering of values due to heterogeneity and to be able to measure prophasic or pseudoprophasic nuclei, we have used the two wave-lengths method of Patau[4] on some of these. This technique is more accurate but slower, and cannot be applied to all nuclei in our conditions. Measurements were made at 485 ± 20 mμ and 515 ± 20 mμ. Results are transformed into the units used above by a correction factor. All the prophasic nuclei measured by this technique in treated cultures proved to be tetraploids, and some very granular nuclei showed similarly high values.

As the treated cultures contain fewer mitotic figures (other than arrested prophases) than the controls, the difference between them is not due to a higher rate of cell multiplication in the treated cultures. Indeed, in an actively growing culture, the histogram has still another shape: a tetraploid peak of up to 15 per cent of cells is found with but few intermediate values. The effect observed is still different from that found in colchicine-treated cultures, where tetra- and octoploid nuclei appear as the result of mitoses interrupted at metaphase by spindle inhibition[5].

It appears that removal of cellular ribonucleic acid by ribonuclease does not prevent the synthesis of deoxyribonucleic acid by the cells before prophase, but inhibits further development of mitosis. We have thus a true prophasic inhibition of mitosis. It can be concluded that ribonucleic acid is not

involved in the synthesis of deoxyribonucleic acid but is in some way necessary to the normal mitosis in this material.

This work, including comparison with other materials, will be reported more fully elsewhere.

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A Critical Method of measuring the Diameter of Living Bacteria with the Interference Microscope

With an ordinary microscope, the accuracy of measurement is limited by the numerical aperture of the optical system and the wave-length of the light used, and it is scarcely possible to determine the dimensions of an object more accurately than to the nearest 0·4μ. Consequently, the size of very small objects such as living bacteria can only be estimated roughly by direct measurement.

With the interference microscope, however, it is possible to measure very critically the amount by which the light is changed in phase as it passes through an object, and, since this phase-change is proportional to the product of the object's refractive index and thickness, it should be possible to measure its thickness with considerable accuracy if its refractive index were known. One would no longer be limited in accuracy by the wave-length of the light used.

It is, in fact, possible to measure the refractive indices of many living bacteria quite accurately by the method of immersion refractometry developed in 1952 by R. Barer and his collaborators[1]: and so an attempt was made to combine this method with interferometry to obtain an accurate estimate of the mean thickness of a living bacillus.

The organism selected was *Bacillus bulgaris*, cultures of which were obtained from the Yalacta Laboratories in Paris. When measured with an eye-piece micrometer, it always appeared to be between 1μ and 1·5μ in diameter, both in the original cultures and in the subcultures grown from them. The refractive indices of all the bacilli were found to lie between 1·4000 and 1·4090 in each of the cultures investigated; and, when suspended in a bovine plasma–albumin solution that had a refractive index of 1·4045, approximately equal numbers of dark and bright bacilli were visible under the phase-contrast microscope. This figure was therefore taken as their mean refractive index.

Bacilli were suspended in a 0·25 per cent sodium chloride solution for examination with the interference microscope. This suspension-fluid was assumed, as a result of a previous investigation[2], to have a tonicity approximately equivalent to the above-mentioned bovine plasma solution, and it had a refractive index of 1·3350. The double-refracting interference microscope of Messrs. Charles Baker of Holborn was used, and measurements were made with a 2-mm. double-focus objective in conjunction with the recently developed half-shade matching eye-piece described by Smith[3]. Light from a 'Pointolite' lamp was used, filtered through an Ilford 807 filter, which gave a narrow green spectrum with a maximum intensity at about 542 mμ. With this arrangement, it was possible to measure the phase-changes caused by bacilli lying with their long axes at right angles to the optical axis of the microscope to the nearest 1/60 wave-length, or more accurately than this.

It was, however, necessary to apply a small correction to these recorded measurements, because the bacilli were too small for any intensity differences to be appreciated between their edge and middle line; which their cylindrical cross-section would lead one to expect. Thus, each phase-change recorded represented the integrated phase-change over the whole object, such as would be caused by its *mean* thickness as opposed to its *maximum* thickness. The mean thickness of an object of circular cross-section is equal to the length of the side of a square equal in area to the circle: and a correction of $\times \frac{2}{\sqrt{\pi}}$ or approximately $\times$ 1·13 must therefore be applied.

Ten measurements of the phase-change were made on different bacilli in each culture studied, and the mean taken. The mean thickness, t, of the bacilli in each preparation was then calculated from the formula:

$$t = 1{\cdot}13 \times \frac{\varphi}{360} \times \frac{\lambda}{n-m}$$

where φ was the measured mean phase-change expressed as an angle, λ the mean wave-length of the light used (542 mμ), n the mean refractive index of the bacilli (1·4045), and m the refractive index of the saline mounting medium (1·3350).

Table 1

Bacillus bulgaris culture (date)	Mean φ (degrees)	t (μ)
Paris, 11/7	55·8	1·37
Paris, 11/7; subcultured, 16/7	55·4	1·36
Paris, 11/7; subcultured, 24/7	52·6	1·29
Paris, 25/7	52·8	1·29
Paris, 25/7	53·2	1·30
Paris, 25/7; subcultured, 27/7	52·2	1·28
Paris, 25/7; subcultured, 27/7	53·6	1·31
Paris, 25/7; subcultured, 27/7	52·0	1·28
Paris, 25/7; subcultured, 27/7	54·4	1·33
Paris, 25/7; subcultured, 27/7	53·6	1·31

Table 1 shows the values for the mean phase-change and thickness obtained in ten different preparations, including cultures and subcultures of several different dates. Each figure in the table is the mean of ten observations. It will be seen that the mean thicknesses thus calculated vary from 1·28μ to 1·37μ: a maximum scatter of only 0·09μ. It is evident that the diameter of the bacilli in the various cultures is remarkably uniform. The limit to the accuracy of this method is presumably set by the accuracy with which φ can be determined.

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Taxonomic Position of the Genus *Selenomonas* (von Prowazek)

In a recent analytical review[1], Lessel and Breed have reiterated the conclusions of Boskamp[2] upon the bacterial nature of *Selenomonas palpitans* (Simons, *ex* Boskamp). Although they do not add materially to the information available, these authors state that *Selenomonas* clearly belongs to the family Spirillaceae.

I have studied the morphology both of *Selenomonas palpitans* from the guinea pig cæcum, and of *S. sputigena* from the human mouth, and am of the opinion that the nuclear structures, flagellation and mode of cell division are entirely unlike those of bacteria and reflect a much closer relationship with the protozoa.

The nucleus consists of a filament with expanded ends, one of which is connected by a rhizoplast to the blepharoplast, from which arises the flagellum, usually at the centre of the concave side of the crescentic cell. The nucleus and a portion of the neuromotor apparatus are embedded in deeply staining basophilic masses, in the form of two cones, point to point (Fig. 1,*b*). When the nucleus divides, a second rhizoplast appears, joining the blepharoplast to the free end of the nuclear filament. The blepharoplast divides, initiating division of the flagellum, and the division of the nucleus follows (Fig. 1,*c*). The cell then divides by constriction. This is entirely unlike any known scheme of nuclear division in bacteria[3].

The cell wall fails to stain in the characteristically bacterial manner with tannic acid and crystal violet, or with phosphomolybdic acid and methyl green[3,4], and the cell lacks the cross-walls which are found in many bacteria, including spirilla[3]. The flagellum, when compared with those of bacteria in the same field of the electron microscope, was entirely distinct in its appearance. It is composed of numerous tiny fibrils, like the flagella of protozoa, and unlike either the monofibrillar flagella of bacteria or the compound flagella of certain spirilla[3]. The flagellum of *Selenomonas* is also distinct from that of bacteria in that it can be demonstrated in the light-microscope by staining with Giemsa or simple dyes without previous mordanting. The frayed 'whip-tuft' appearance shown by Boskamp[2] and others is an artefact, due to unsuitable technique. The cell moves in an irregular tumbling course, which once more is more reminiscent of protozoa than of bacteria.

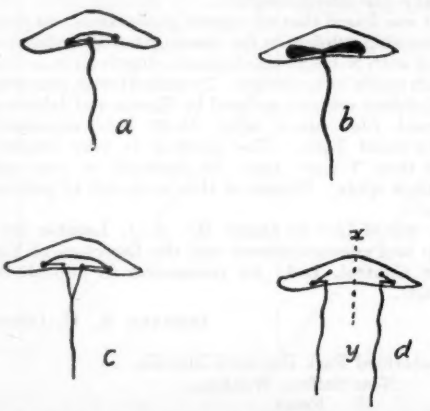

Fig. 1. (*a*) Nuclear structure in an adult cell of *S. palpitans*. (*b*) Masking basophilic materials often confused with the nucleus proper. (*c*) Division of nucleus and splitting of flagellum. (*d*) Nuclear division completed, cell now divides by constriction along *x*—*y*

These findings indicate clearly that the affinities of the genus *Selenomonas* are with the Protozoa. The classification of Wenyon[5], which places it in the family Monadidae (Kent), is adequate to the present extent of our information.

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Paper Chromatographic and Biological Properties of Reserpine and Related Compounds

Little is known of the metabolism (in man and experimental animals) of the alkaloids of *Rauwolfia serpentina*. We are particularly interested in the reported antifertility effects of reserpine in rats[1] and the mechanisms of sedative action[2] of *Rauwolfia serpentina* alkaloids in schizophrenic patients. Reserpine is known[3] to be hydrolysed *in vivo* to methyl reserpate and presumably trimethoxybenzoic acid. The latter compound has been shown[4] to influence certain enzyme systems *in vitro* which are of importance in relation to the mode of action of œstrogens on the uterus. Using a fertility performance test, we have studied the effects of the administration to male and female rats of daily oral doses of 80–100 mgm. of sodium trimethoxybenzoate given over a period of ten weeks. Since no alteration in fertility was observed, we conclude that reserpine itself or some polycyclic metabolite is responsible for the observations of previous workers[1].

We recommend the following paper chromatographic procedures as a means of checking the purity of commercial samples of *Rauwolfia* alkaloids and determining the composition of complex mixtures of the alkaloids used for therapeutic purposes and in pharmacological tests. At the time of writing, we are unaware of any detailed reports on paper chromatography of *Rauwolfia* alkaloids. The methods introduced by us employ Whatman paper No. 542 and the following developing solvent systems:

(1) *'Single phase' systems* (no pre-equilibration, capillary ascent technique employed).

(*a*) 10 per cent (v/v) acetic acid in aqueous 5 per cent sodium acetate is shaken with *n*-butyl ether, added in small portions, until saturation of the aqueous phase is just reached. The clear aqueous phase is used.

(*b*), (*c*) and (*d*), similar to (*a*) but using tertiary amyl alcohol, *sec.*-butyl alcohol or methyl*iso*butylketone instead of *n*-butyl ether. (*d*) was particularly useful for slow-moving components in (*a*). Similar solvents have been used previously for urinary phenols and œstriol[5].

(*e*) Xylene 200, methanol 75, methyl*iso*butylketone 25.

(2) *Partition chromatography* (pre-equilibration required).

(*a*) *n*-Amyl alcohol 200, water 180, acetic acid 20.

(*b*) *n*-Hexyl ether 200, methyl*iso*butylketone 50, acetic acid 20, water 180.

Two-dimensional paper chromatography of a commercial concentrate of reserpine and other alkaloids from *Rauwolfia serpentina* revealed twenty-four fluorescent components without using any spray reagents. Suitable combinations of solvents were (2*b*) followed by (1*a*) or (1*b*), or (1*e*) followed by (1*a*).

The following tests may be used for locating the compounds: (i) Fluorescence in ultra-violet light (Wood's glass filter). (ii) Fluorescence after streaking or spotting the paper with 3 per cent sodium nitroprusside in 50 per cent trichloracetic acid (cf. ref. 3). (iii) Fluorescence 30 min. after spotting the paper with microdroplets of 90 per cent sulphuric acid and later spraying with 9 per cent sodium carbonate. Intense blue, green or greenish-blue fluorescent spots are obtained in the alkaloid zones. (iv) Tests (ii) and (iii) followed by spraying with alkaline diazotized I.C.I. 5091 [5]—pink colours. Methyl reserpate and ajmaline are particularly intense. (v) Ehrlich's dimethylaminobenzaldehyde reagent; ajmaline-blue spot which is intensified by the reagent used in (ii). (vi) Bismuth iodide – iodine in ethyl acetate, acetic acid followed by 0·5 per cent sodium dithionite to remove the orange background. Several compounds give salmon-coloured spots; but the sensitivity is not high.

Tests (i) to (iv) are used together as a routine on single chromatograms.

Solvent system (1*a*) gave the best results and tests (ii) and (iii) gave positive reactions with less than 1 μgm. amounts of reserpine and rescinnamine.

The following relative R_F values were obtained using solvent (1*a*): ajmaline 0·76, deserpidine 0·41, methyl reserpate 0·52 principal spot, and 0·76 as well as two other minor components, rauwolscine 0·63, reserpine 0·34, rescinnamine 0·26, serpentine 0·5, yohimbine 0·58, new yohimbine 0·62. With solvent (1*b*) R_F values were: ajmaline 0·75, ajmalicine 0·5, canescine 0·59, corynanthine 0·64, reserpine 0·43, and impurity 0·55, rescinnamine 0·31, and impurity 0·41, serpentine 0·5.

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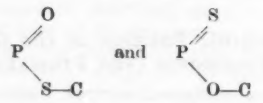
A General Method for locating the Spots on a Paper Chromatogram of Tertiary Alkyl Phosphates, Thiolophosphates and Thionophosphates

There is no method described in the literature that is generally satisfactory for rendering these compounds visible in paper chromatography. Hanes and Isherwood[1] describe a method for phosphoramides, for example, 'Schradan'. This method fails with many compounds containing



In the case of 'Systox', about 50 μgm. gives positive results when treated with alkali for a period of up to 70 hr.

J. W. Cook[2] reported a new spot test for some organo-phosphorus compounds containing sulphur ('Systox' and its isomers). In this method the chromatogram is first treated with N-bromosuccinimide and then with fluorescein. The former reacts with the sulphur-containing organo-phosphorus compound. The remaining area of the paper contains free N-bromosuccinimide which afterwards reacts with fluorescein, forming a non-fluorescent compound. Exposing the chromatogram to ultra-violet light renders the organo-phosphorus compound visible as a fluorescent spot. Only when the proper amounts of N-bromosuccinimide and fluorescein are used is the bromine completely absorbed by the spot, which is then located by the fluorescence of fluorescein. This method has therefore the disadvantage that a controlled amount of bromine and fluorescein has to be used. If a quantity of N-bromosuccinimide greater than is needed to brominate the compound is used, the excess will brominate the fluorescein as in the background, and thus mask the spot. Similarly, an excess of fluorescein will mask the spot through fluorescence of the background.

If there is more than one spot and the quantities of substance in the spots differ, it follows that it is impossible to adjust the amounts of reagents to reveal them all and give an indication of their concentration in any one chromatogram.

It was found that all organo-phosphorus compounds containing sulphur so far investigated are, after treatment with N-bromosuccinimide (dissolved in acetone), much easier to hydrolyse. Treatment with ammonium molybdate solution as used by Hanes and Isherwood[1] formed blue spots after 10–20 min. exposure to ultra-violet light. The method is very sensitive; less than 7 μgm. may be detected as very clear, distinct spots. Details of this work will be published later.

I would like to thank Mr. A. J. Lambie for his help and encouragement and the Directors of Fisons Pest Control, Ltd., for permission to publish these results.

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[1] Hanes and Isherwood, *Nature*, **167**, 1107 (1949).
[2] Cook, J. W., *J. Assoc. Off. Agric. Chem.*, **37**, No. 4, 984 (1954).

Growth of Pre-Breakdown Ionization Currents in Hydrogen

THE growth of ionization currents with distance at constant low values of E/p (E is the field, p the gas pressure) at high values of p has been investigated in this laboratory primarily to obtain precise determinations of the ionization coefficients α and ω/α in conditions when special attention was paid to the state of the gas and cathode surface. Some of the results obtained preliminary to the main part of this investigation are, however, of general interest.

The ionization chamber used was the same as that in similar work with air[1] and nitrogen[2]; the gas was tank hydrogen passed through heated palladium and afterwards cooled by liquid air. A typical $\log I/I_0$ vs. d curve (I is the ionization current, I_0 the initial current from the cathode, d the gap distance) for E/p = 21 V./cm. mm. mercury and p = 220 mm. mercury is shown in Fig. 1, which also gives for comparison a curve obtained for hydrogen by Ayres[3] for E/p = 73·5 V./cm. mm. mercury at the low pressure, p = 4·1 mm. mercury. It can be shown that both curves agree within the experimental error with the well-known Townsend relationship:

$$I = I_0 \exp \alpha d/\{1 - \omega/\alpha (\exp \alpha d - 1)\}$$

where α is the primary ionization coefficient and ω/α a generalized secondary ionization coefficient. It follows from this result that the mechanism which sets the criterion for static breakdown in uniform fields is the same at $pd_s \sim 640$ mm. mercury cm. at a pressure of 220 mm. mercury as at $pd_s \sim 10$ mm. mercury cm. at a pressure of 4·1 mm. mercury (d_s being the sparking distance). In this respect the electrical properties of hydrogen are similar to those of air and nitrogen.

Other values of pd_s have been investigated and similar results obtained; these results will be discussed elsewhere, since they are concerned mainly with the precision determination of the coefficients and cathode effects. However, since previous measurements[3–5] of α/p were mostly obtained at low gas pressures and there are considerable discrepancies between results obtained by different observers, it is of interest to give here the preliminary values

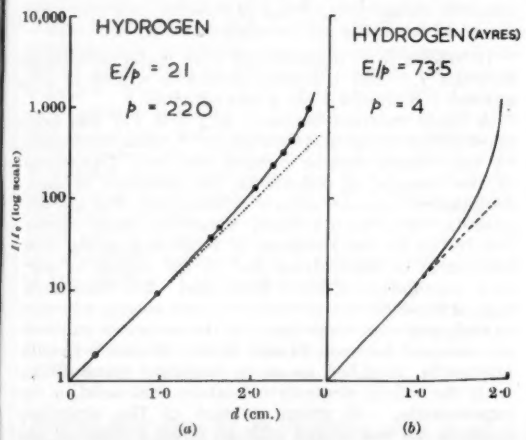

Fig. 1. Curves of $\log I/I_0$ vs. d for hydrogen. (*a*) High pressure: E/p = 21 V./cm. mm. mercury; p = 220 mm. mercury. (*b*) Low pressure (ref. 3): E/p = 73·5 V./cm. mm. mercury; p = 4 mm. mercury

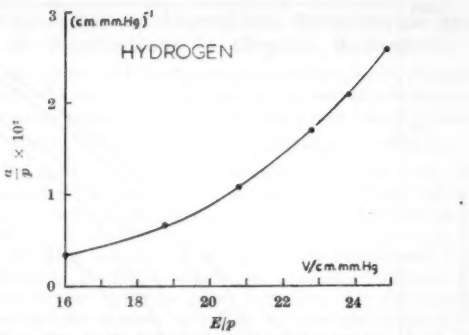

Fig. 2. α/p vs. E/p in hydrogen at a pressure of 150 mm. mercury

(maximum error ~ 15 per cent) obtained for α/p. These values, obtained for a range of E/p from 16 to 25 V./cm. mm. mercury at a pressure of 150 mm. mercury, are shown in Fig. 2. There is good agreement between these results and the recent results obtained by Rose[6] using modern techniques for the same range of E/p but at low gas pressures. This agreement in values of α/p obtained at the same E/p but widely different gas pressures is direct experimental evidence that the similarity relation $\alpha/p = f(E/p)$ holds for primary ionization over a wide range of pressures from pressures of a few millimetres to 150 mm. mercury in hydrogen. It follows, therefore, that the mechanism of primary ionization is the same over this range of pressures, and implies that the applied field is not substantially distorted in the electron avalanche with the initial current densities $\sim 3 \times 10^{-12}$ amp./cm.^{2} used.

We wish to thank the Central Electricity Authority for its support of this investigation; and we are grateful to Prof. F. Llewellyn Jones, in whose laboratory this work was carried out, for his help and interest.

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Measurements of Retarding Potential on the Hollow Cathode

THE hollow cathode[1] consists of a cylindrical closed box of nickel with a small hole at one end. The inner surfaces of the box and the hole are coated with conventional cathode paste.

Various attempts have been made to explain the high current densities (10–100 amp./cm.^{2}) which can be obtained from such a cathode; but these cannot be verified until the experimental investigation of the cathode has been completed. In particular, no

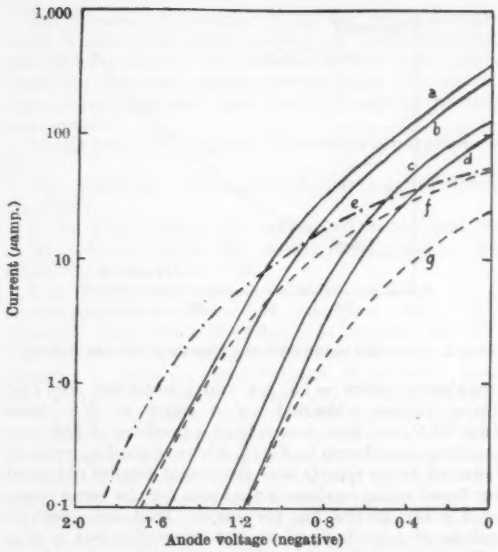

Fig. 1. ———, Hollow cathode: (*a*) 940° C., (*b*) 920° C., (*c*) 865° C., (*d*) 840° C. – – – –, 'Plug' cathode: (*f*) 940° C., (*g*) 840° C. ——·——, Flat cathode: (*e*) 940° C.

information was available on the temperature of electrons leaving the cathode, as determined by retarding potential measurements. Such measurements have now been made on hollow cathodes of two types and these have been compared with results obtained with planar diodes.

One type of hollow cathode had an end-wall thickness of 0·75 mm. and a hole diameter of 0·35 mm., and was placed 1·9 mm. away from a plane molybdenum anode. The second type was a 'plug' cathode geometrically similar, but with the hole completely filled with cathode paste instead of being merely coated on its sides. For comparison purposes, some planar diodes were made with a cathode diameter of 4 mm. Typical results are shown in Fig. 1.

It is interesting to compare the curves for the 'plug' and 'hollow' cathodes. The effect of filling the hole in the hollow cathode with cathode paste was to reduce the number of slow electrons emerging from the cathode. The curves tend to merge together as the anode becomes more negative. It was not possible to estimate the electron temperature accurately from the slope of log I plotted against $-V$ because the retarding potential plots did not give the expected straight lines, even with planar cathodes. The electron temperatures calculated from the linear portion of the graph for the hollow cathode were about 1·4 times the values of cathode temperature found from pyrometer and thermocouple measurements. A similar discrepancy was noted in the case of the flat oxide-coated cathode, and this is in agreement with results obtained by Nottingham[2]. He found that the phenomenon was peculiar to oxide-coated cathodes and did not occur with thermionic emission from pure metals.

For low values of anode voltage there was an apparent deficiency in slow electrons for all three types of cathode. It is interesting to note that the effect was more pronounced with the hollow cathode than with the 'plug' cathode. Similar results were obtained with conventional oxide cathodes by Reynolds[3].

The results tend to suggest that the electron temperature of the hollow cathode does not differ greatly from that of a normal oxide cathode at the same cathode temperature. The explanation of the high current densities observed with positive anode voltage does not appear to be due to the property of the electron gas inside the box. Tests on the 'plug' cathode showed that it was possible to draw anode currents of the same order as those obtained with the hollow cathode.

These measurements are indirect support of the hypothesis that the greater part of the emission comes from coating near the edge of the hole.

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A. W. Bright
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Services Electronics Research Laboratory,
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[1] *Nature*, **174**, 1176 (1954).
[2] Nottingham, W. B., *Phys. Rev.*, **41**, 793 (1932).
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Electrophoretic Measurement of the True Zeta-Potential of Arsenious Sulphide Sol in the Region of Slow Coagulation

According to Bikerman[1], for "rod-shaped particles with half spherical ends" the true ζ-potential and ζ_a, the potential calculated from the Smoluchowski equation, are linked by the following equation

$$\zeta = \zeta_a \left[1 + \frac{2\chi}{(L-r)S}\right] \qquad (1)$$

where χ represents the specific surface conductivity, S the bulk specific conductivity of the intermicellar fluid, L the length and r the radius of the cross-section of the particle. The equations of Henry[2] and Booth[3] for spherical particles and sufficiently large value of the product of the radius of the particle and the reciprocal of the thickness of the electrical double layer can be expressed in the form

$$\zeta = \zeta_a \left(1 + \frac{\chi}{rS}\right) \qquad (2)$$

$$\text{or } \frac{1}{\zeta_a} = \frac{1}{\zeta} + \frac{\chi}{\zeta r S} \qquad (3)$$

It follows from equation (3) that under conditions in which χ, r and ζ remain constant, a plot of $1/\zeta_a$ against $1/S$ should yield a straight line.

A linear relation between $1/\zeta_a$ and $1/S$ has been observed by Ghosh and co-workers[4–6], when measuring by the electro-osmotic method the true ζ-potential of the coagula of colloids in the presence of equi-coagulating concentration of electrolytes. The electrophoretic experiments have, therefore, been carried out by us in the presence of equi-coagulating concentration of electrolytes and in the region of very slow coagulation of the colloid used. The concentration of the different electrolytes used was so adjusted in each case that coagulation of the arsenious sulphide sol occurred between 24 and 25 hr. Burton's U-tube apparatus, modified so as to facilitate quick filling with the colloid electrolyte mixture, was used in the experiments. A given volume of the arsenious sulphide sol was mixed with an equal volume of the electrolyte of proper concentration and allowed to stand for 30 min. It was next centrifuged for 3 min. at a given speed of rotation, the upper portion of the

Table 1

Electrolyte used	$S \times 10^4$ in mhos	Values of ζ_e for the movement of boundary		Average ζ_e in millivolts
		Upward	Downward	
KCl	75·60	44·32	48·48	46·4
$BaCl_2$	8·70	23·83	26·63	25·2
$MgCl_2$	8·87	26·03	26·20	26·1
$AlCl_3$ (containing a little HCl)	6·75	31·98	32·08	32·0
$AlCl_3$ (containing less HCl)	5·59	31·07	32·22	31·6
$AlCl_3$ and KCl mixture	10·53	37·16	37·20	37·2
$BaCl_2$ and KCl mixture	12·27	30·42	30·60	30·5
$BaCl_2$ and KCl mixture	15·10	33·05	32·25	32·7

sol was separated from the sediment of large particles and the lower part of the *U*-tube was filled with it, the upper part being filled with a solution having the same specific conductivity as that of the intermicellar fluid of the sol occupying the lower part. The electric current, i, flowing through the system during electrophoresis was carefully measured. From the cross-section of the *U*-tube, the current, i, and the specific conductivity of the intermicellar fluid, the potential gradient was calculated. (In no case did the specific conductivity of the intermicellar fluid differ from that of the corresponding colloid–electrolyte mixture by more than 4 per cent. In the case of potassium chloride the difference was less than 1 per cent.) The results are recorded in Table 1.

From these results it was found that if $1/\zeta_e$ is plotted against $1/S$, two straight lines are obtained, one for the mixtures of aluminium chloride with hydrochloric acid and potassium chloride, and the other for magnesium chloride, barium chloride and mixtures of barium chloride and potassium chloride. These lines meet the $1/\zeta_e$ axis at two points very close to each other, giving the average value of $\zeta = 49 \pm 1$ mV. The different values of $\chi/\zeta r$ for the two straight lines are therefore to be attributed to the variation of either χ or r, or both, depending on the nature of the electrolytes used. Electro-osmotic data obtained with negatively charged silver iodide coagula[5] indicate that χ does not depend on the nature of the electrolytes, and we are inclined to attribute the variation of $\chi/\zeta r$ to the change of r only.

The true ζ-potential found for the same arsenious sulphide sol under identical concentrations of the stated electrolytes by the electro-endosmotic method is 51 mV. The values of the true ζ-potential of arsenious sulphide particles in the region of slow coagulation in the presence of equicoagulating concentrations of the electrolytes, as measured by the electrophoretic and electro-osmotic methods, are thus in good agreement, and their mean is 50 mV. These results, therefore, confirm our previous conclusion that a given rate of coagulation of a colloid is characterized by a definite value of the ζ-potential independent of the valency of the counter ions used.

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[1] Bikerman, *Trans. Farad. Soc.*, **35**, 154 (1940).
[2] Henry, *Trans. Farad. Soc.*, **44**, 1021 (1948).
[3] Booth, *Trans. Farad. Soc.*, **44**, 455 (1948).
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Preparation of Aluminium Borohydride and its Applications in Organic Reductions

It was found that the reaction of aluminium chloride with calcium borohydride[1] in a tetrahydrofuranic solution gives a solution of aluminium borohydride:

$$2AlCl_3 + 3Ca(BH_4)_2 = 2Al(BH_4)_3 + 3CaCl_2.$$

Calcium chloride is precipitated and can be separated by centrifuging in sealed centrifuge tubes; the supernatant contains the tetrahydrofuranic adduct of aluminium borohydride. (By cooling to $-50°$ this solution gives colourless crystals of this adduct.) Partial evaporation of such a solution at atmospheric pressure gives a residue the aluminium borohydride content of which is considerably greater. Since the boiling point of aluminium borohydride is about 20° lower than that of tetrahydrofurane, this shows that the aluminium borohydride adduct has considerable stability.

The tetrahydrofurane solution of aluminium borohydride is not spontaneously inflammable in air (unlike undiluted $Al(BH_4)_3$), and thus it is relatively safely applicable for reductions of organic compounds.

It is interesting to note that aluminium chloride gives a good yield of the borohydride only with calcium borohydride; under similar conditions (tetrahydrofurane solution) lithium borohydride gives a small yield, while sodium borohydride does not react.

With such solutions (or with solutions of distilled aluminium borohydride[2] in dry tetrahydrofurane) carboxylic acid esters can be reduced selectively to the corresponding primary alcohols. For example, ethyl benzoate and ethyl-*p*-nitrobenzoate are at room temperature reduced to the corresponding carbinols; similarly the aldehydes and ketones, respectively.

Nitriles give the corresponding primary amines: benzyl cyanide and 3,4-dimethoxy benzyl cyanide give primary amines with a yield of about 50–60 per cent.

Nitriles are not reduced with the borohydrides of the alkaline metals; even calcium borohydride—which is a much stronger reducing agent—reduces nitriles only very slowly and with low yields.

Thus the reducing power of the complex borohydrides is lowest in the class of the borohydrides of the alkaline metals (which have a structure of ionic salt character) and highest in the case of aluminium borohydride, which represents a typical covalent structure (boiling point 44° (760 mm.)). The alkaline-earth metal borohydrides represent an intermediate grade in structure as well as in their reducing power.

These correlations between structure and reducing power have, in our opinion, a bearing on the mechanism of such reductions and indeed point to the importance of a molecular reducing mechanism.

Note added in proof. After submitting this paper, a communication appeared by H. C. Brown and B. C. Subba Rao (*J. Amer. Chem. Soc.*, 77, 3164; 1955) dealing with applications of a solution of sodium borohydride and aluminium chloride in diethylene glycol-dimethyl-ether for reduction of organic compounds.

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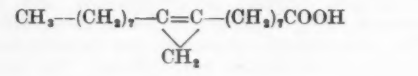
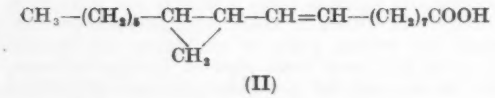
[1] Kollonitsch, J., Fuchs, O., and Gábor, V., *Nature*, **175**, 346 (1955).
[2] Schlesinger, H. J., Brown, H. C., and Hyde, E. K., *J. Amer. Chem. Soc.*, **75**, 209 (1953).

Structure of Sterculic Acid

In 1952, Nunn[1] put forward a structure for sterculic acid (I), whereas we have assigned to it structure (II)[2]; Dijkstra and Duin[3] have, however, rejected structure (II) on grounds which we believe to be inconsistent with the known facts.



(I)



(II)

(1) Derfer *et al.*[4] have found that the derivatives of *cyclo*propane exhibit an infra-red absorption band in the region 1,000–1,020 cm.$^{-1}$; consequently, they have suggested that a band near 1,010 cm.$^{-1}$ may be used for detecting the presence of a *cyclo*propyl group in a compound.

A *cyclo*propane ring conjugated with a C=C bond (vinyl-*cyclo*propane) has been found by Van Volkenburgh *et al.*[5] to possess a band at 1,010 cm.$^{-1}$. As our value for sterculic acid is 1,004 cm.$^{-1}$ (our revised value[6] being 1,008 cm.$^{-1}$), which is very close to the value of the conjugated compound cited above, a similar type of structure is not unlikely in sterculic acid. Recent work carried out in this laboratory[6] has shown that the bands of the similar *cyclo*propane and *cyclo*propene derivatives prepared synthetically lie almost in the same region, and no analytical value could be set for characterizing a *cyclo*propene group.

(2) Besides cotton seed oil, the Halphen reaction is given by many other seed oils, which according to their reported analyses do not contain even a trace of any fatty acid having a *cyclo*propene group. Further, the exact mechanism of the reaction and nature of the chromogenic substance are not yet settled; possibly some component of the non-saponifiable matter present in the oil is concerned with this colour reaction. In view of this, it is difficult to accept that the reaction is due to the *cyclo*propene grouping alone.

(3) The view that a *cyclo*propane group is less reactive than an ethylene bond is not corroborated in many cases[7]. *Cyclo*propane and its derivatives have been reported[8] to possess appreciable instability and resemble closely the corresponding olefinic compounds in their reaction to heat and some reagents. Thus there does not seem to be any marked fundamental difference between the corresponding derivatives of *cyclo*propane and ethylene, and a *cyclo*propane ring conjugated with a C=C or any other classically unsaturated group behaves very similarly to a typical diene conjugated system[7,9]. It has also been reported[9] that *cyclo*propane and *cyclo*butane are unstable relative to the isomeric alkenes.

On the other hand, the *cyclo*propene derivatives are not so unstable as Dijkstra and Duin[3] have indicated. Their comparative stability and semi-aromatic character were demonstrated by Goss, Ingold and Thorpe[10] so early as 1923. Further, the work carried out in this laboratory[6] on the syntheses of *cyclo*propene compounds has shown that they are fairly stable, so much so that they could be vacuum-distilled without decomposition. It may be added that the comparative study of the polymerization of sterculic acid and 9 : 11-octa-decadienoic acid has not so far been reported, but it is well known that dehydrated castor oil, in which the glyceride of the latter is present to the extent of only 25 per cent, gels at ordinary temperature after some time.

Finally, a strong argument against the structure (I) proposed by Nunn[1] is that the compound of this constitution has been synthesized and proved to be different from sterculic acid as already reported[2,6].

Dijkstra and Duin[3] have isolated an unknown C_{18}-fatty acid from kapok seed oil and assume it to contain a *cyclo*propene group, thus leaving a straight chain of 17-carbon atoms (odd number); this is unique and merits careful investigation.

The chemical and analytical data giving further evidence for structure II will shortly be published elsewhere.

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Sept. 14.

[1] Nunn, J. R., *J. Chem. Soc.*, 313 (1952).
[2] Verma, J. P., Nath, Bhola, and Aggarwal, J. S., *Nature*, 175, 84 (1955).
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Humidity and Corrosion

I should like to support and emphasize Dr. J. C. Hudson's comments on Major P. A. Cartwright's communication in *Nature* of October 15, p. 748. As the figures (obtained at the Ministry of Supply Tropical Testing Establishment, Nigeria; see Ambler and Bain, *J. App. Chem.*, **5**, 437 (1955)) in the table below show, corrosion at two sites of roughly equal humidity may range from 'highest anywhere' to less than in rural Britain, the operative variant being salinity.

	R.H. (per cent)		Corrosion of mild steel (gm./dm.^{2}/month)	Salinity (sodium chloride deposited on damp surface: mgm./m.^{2}/day)
	Av. max.	Av. min.		
Lighthouse Beach, Lagos, 50 yd. from H.W.M.	94	78	5·6	800
Jungle clearing at Nkpoku, 30 miles from sea	95	80	0·10	7·5

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FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, December 5

NUTRITION SOCIETY (at the Royal Society of Medicine, 1 Wimpole Street, London, W.1), at 5 p.m.—Dr. Harry J. Deuel, Jr. (U.S.A.): "Fat as an Essential Component of Diet".

Tuesday, December 6

INSTITUTION OF CHEMICAL ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. E. S. Sellers and Mr. D. R. Augood: "Distillation of Liquid Hydrogen"; Mr. J. F. Davidson: "Transient Behaviour of Plate Distillation Columns".

UNIVERSITY OF LONDON (at the Royal College of Science, South Kensington, London, S.W.7), at 5.30 p.m.—Prof. A. Tustin: "The Route to Human Welfare" (Inaugural Lecture).

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (in the Rooms of the Chemical Society, Burlington House, Piccadilly, London, W.1), at 6.30 p.m.—Dr. M. Gordon: "Second Order Transitions in High Polymers".

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. W. A. P. Fisher: "Fatigue Aspects of Structural Design".

SOCIETY OF INSTRUMENT TECHNOLOGY, CONTROL SECTION (at Manson House, Portland Place, London, W.1), at 7 p.m.—Mr. Eric B. Pearson: "Planning a Servo-mechanisms Laboratory for Instructional Purposes".

SOCIETY FOR VISITING SCIENTISTS (at 5 Old Burlington Street, London, W.1), at 7.30 p.m.—Discussion Meeting—"Government Responsibility in Industrial Research". Chairman: Sir Henry T. Tizard, F.R.S. Speakers: Dr. E. W. R. Steacie, F.R.S., and Dr. B. K. Blount.

Wednesday, December 7

ROYAL SOCIETY OF ARTS (at John Adam Street, Adelphi, London, W.C.2), at 2.30 p.m.—Mr. H. Bagenal: "Planning Against Noise" (Alfred Bossom Lecture).

EUGENICS SOCIETY (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Prof. H. J. Eysenck: "The Nature and Inheritance of Extraversion".*

INSTITUTE OF FUEL (at the Institution of Civil Engineers, Great George Street, London, S.W.1), at 5.30 p.m.—Dr. Alfred Parker, C.B.E.: "Power, Population and Productivity" (Melchett Lecture).*

INSTITUTION OF ELECTRICAL ENGINEERS RADIO AND TELECOMMUNICATION SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. S. Webley, Dr. H. G. Lubczynski and Mr. J. A. Lodge: "Some Half-Tone Storage Tubes".

INSTITUTE OF METALS (at 4 Grosvenor Gardens, London, S.W.1), at 6.30 p.m.—"Metals at Low Temperatures", a Report on the recent Low-Temperature Conference held in Paris. Speakers: Dr. P. L. Smith and Dr. H. M. Rosenberg, introduced by Sir Francis Simon, F.R.S.*

INSTITUTE OF PHYSICS, LONDON AND HOME COUNTIES BRANCH (at 47 Belgrave Square, London, S.W.1), at 6.30 p.m.—Sir Geoffrey Taylor, F.R.S.: "Recent Studies of Water Waves".

Wednesday, December 7—Thursday, December 8

INSTITUTE OF WELDING (joint meeting with the BRITISH WELDING RESEARCH ASSOCIATION, INSTITUTION OF NAVAL ARCHITECTS, BRITISH SHIPBUILDING RESEARCH ASSOCIATION and the ALUMINIUM DEVELOPMENT ASSOCIATION, in the Weir Lecture Hall, Institution of Naval Architects, 10 Upper Belgrave Street, London, S.W.1), at 10 a.m. daily—Symposium on "The Use and Welding of Aluminium in Shipbuilding".

Thursday, December 8

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Discussion on "Dynamics of Natural Populations" opened by Dr. E. B. Ford, F.R.S.

BRITISH PSYCHOLOGICAL SOCIETY, INDUSTRIAL SECTION (in Room 424, Birkbeck College, Malet Street, London, W.C.1), at 1 p.m.—Dr. E. P. Kelsall: "Strikes from the Viewpoint of a Psychologist".

ROYAL SOCIETY OF ARTS, COMMONWEALTH SECTION (at John Adam Street, Adelphi, London, W.C.2), at 5.15 p.m.—Dr. R. S. F. Hennessey: "Some Social Effects of Tropical Medicine within the Commonwealth".

ROYAL METEOROLOGICAL SOCIETY (in the Conference Hall, County Hall, London, S.E.1), at 5.30 p.m.—Mr. P. J. Meade: "Fog and Atmospheric Pollution" (Lecture open to sixth-form scholars).

UNIVERSITY OF LONDON, BRITISH POSTGRADUATE MEDICAL FEDERATION (at the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.1), at 5.30 p.m.—Dr. Audrey Smith: "Experimental Hypothermia in Animals". (Thirteenth of fifteen Lectures on "The Scientific Basis of Medicine".)*

UNIVERSITY OF LONDON INSTITUTE OF EDUCATION (in the Assembly Hall, Institute of Education, Malet Street, London, W.C.1), at 5.30 p.m.—Dr. T. W. Taylor: "Education in Eastern Germany".*

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at the Medical Society of London, 11 Chandos Street, Cavendish Square, London, W.1), at 6.15 p.m.—Scientific Papers.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the BATTERSEA POLYTECHNIC CHEMICAL SOCIETY, at the Battersea Polytechnic, Battersea Park Road, London, S.W.11), at 7 p.m.—Prof. Sir Eric Rideal, F.R.S.: "Surface Chemistry".

BRITISH GLACIOLOGICAL SOCIETY (at Bedford College, Regent's Park, London, N.W.1), at 7.30 p.m.—Annual General Meeting, followed by a discussion on "The Future Lines of Progress in Glaciology".

ROYAL SOCIETY OF TROPICAL MEDICINE AND HYGIENE (at Manson House, 26 Portland Place, London, W.1), at 7.30 p.m.—Dr. P. H. Abbott: "Mycetoma in the Sudan".

SOCIETY OF COSMETIC CHEMISTS OF GREAT BRITAIN (at the Royal Society of Arts, John Adam Street, London, W.C.2), at 7.30 p.m.—Mr. A. J. Krajkeman: "Some Aspects of Perfumery Chemicals".

Thursday, December 8—Friday, December 9

BRITISH INSTITUTE OF RADIOLOGY, incorporated with the RÖNTGEN SOCIETY (at Caxton Hall, Victoria Street, London, S.W.1)—Seventeenth Annual Congress and Exhibition.

Friday, December 9

ASSOCIATION OF APPLIED BIOLOGISTS (in the Meeting Room of the Royal Entomological Society, 41 Queen's Gate, London, S.W.7), at 10.50 a.m.—Scientific Papers.

ROYAL ASTRONOMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Scientific Meeting.

PHYSICAL SOCIETY, ACOUSTICS GROUP (in the Physics Department of Imperial College, Imperial Institute Road, London, S.W.7), at 5.30 p.m.—Mr. E. J. Evans: "The Absorption of Sound in Humid Air".

ASLIB (at the Royal Society of Arts, 8 John Adam Street, Adelphi, London, W.C.2), at 6 p.m.—Discussion on "Information Bulletins, their Contents and Function".

INSTITUTION OF ELECTRICAL ENGINEERS, EDUCATION DISCUSSION CIRCLE (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion on "Aids to Teaching the Subject of Windings in Rotating Machinery".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (in the Chemistry Lecture Theatre, King's College, Strand, London, W.C.2), at 7 p.m.—Dr. F. R. Smith: "Recent Work on the Tropane Alkaloids".*

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Sir Lawrence Bragg, F.R.S.: "The Discovery of Useful Electricity".

Saturday, December 10

BRITISH MYCOLOGICAL SOCIETY (in the rooms of the Linnean Society of London, Burlington House, Piccadilly, London, W.1), at 12 noon—Annual General Meeting.

BRITISH PSYCHOLOGICAL SOCIETY (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 2.30 p.m.—Dr. Robert Leeper: "Relationships of Learning Theory and Personality".

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Mr. W. F. Grimes: "The Science of Archæology".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

HEAD (with high academic qualifications and teaching experience together with industrial or research experience in applied mathematics) OF THE DEPARTMENT OF MATHEMATICS—The Registrar, College of Technology, Suffolk Street, Birmingham 1 (December 7).

RIVER INSPECTOR (preferably Corporate Member of the Royal Institute of Chemistry and the Institute of Sewage Purification), with the Banff, Moray and Nairn River Purification Board—The Clerk to the Board, County Buildings, Elgin (December 8).

LECTURER (Grade II) IN PLANT PATHOLOGY in the Faculty of Agriculture, University College, Ibadan—The Secretary, Inter-University Council for Higher Education Overseas, 1 Gordon Square, London, W.C.1 (December 10).

LECTURERS (with M.R.C.V.S.) IN (*a*) BACTERIOLOGY and (*b*) BIOCHEMISTRY, in the Faculty of Agriculture, University College of the Gold Coast—The Secretary, Inter-University Council for Higher Education Overseas, 1 Gordon Square, London, W.C.1 (December 10).

LECTURER IN GEOGRAPHY AND ECONOMICS—The Principal, Wigan and District Mining and Technical College, Wigan (December 12).

LECTURER IN MATHEMATICS at the University of New England, Armidale, New South Wales, Australia—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 12).

JUNIOR RESEARCH FELLOW, and a SENIOR RESEARCH FELLOW (physicists or physical chemists) IN THE DEPARTMENT OF GLASS TECHNOLOGY—The Registrar, The University, Sheffield (December 15).

NUFFIELD RESEARCH FELLOW at the Wheatstone Physics Laboratory, to work on the physical chemistry of fibrous proteins—Prof. J. T. Randall, F.R.S., Department of Physics, King's College, Strand, London, W.C.2 (December 15).

ASSISTANT LECTURER IN THE DEPARTMENT OF APPLIED MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (December 16).

Head (with high academic qualifications, preferably an honours degree in mining, practical mining experience and teaching experience) of the Mining and Geology Department—The Principal, Wigan and District Mining and Technical College, Wigan (December 19).

Lecturer (graduate in science or technology and preferably with experience of reinforced concrete design) in Structural Engineering—The Registrar, College of Technology, Manchester 1 (December 19).

Lecturer in the Department of Physics—The Registrar, The University, Manchester 13 (December 19).

Chemist, Senior Research Officer or Principal Research Officer grade (with a good honours degree in chemistry, with postgraduate research experience in the fields of inorganic or physical chemistry), with the Australian Atomic Energy Commission, to undertake research in the chemistry of reactor materials including their behaviour under conditions of high temperature irradiation—The Secretary, Australian Atomic Energy Commission, G.P.O. Box 5343, Sydney, Australia (December 31).

Demonstrator (with a good honours degree in physiology, biochemistry or chemistry, with subsidiary physiology, or medically qualified) in Chemical Physiology—The Registrar, The University, Manchester 13 (December 31).

Head (with high academic qualifications and teaching experience together with industrial or research experience in applied physics) of the Department of Physics—The Registrar, College of Technology, Suffolk Street, Birmingham 1 (December 31).

Physicist, Principal Research Officer grade (with a good honours degree in physics, plus at least ten years research experience), with the Australian Atomic Energy Commission, to work in the field of reactor physics embracing such aspects as exponential and zero energy experiments and criticality and kinetic studies—The Secretary, Australian Atomic Energy Commission, G.P.O. Box 5343, Sydney, Australia (December 31).

Plant Physiologist, Statistician (Biometrician), and an Assistant Entomologist in the Wattle Research Institute of the University of Natal, Pietermaritzburg, South Africa—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (South Africa, December 31).

Senior Lecturer in the Department of Mechanical Engineering at the University of Cape Town—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (January 6).

Reader in Mechanical Engineering at King's College, London, W.C.2—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 10).

Reader in Metallurgy at King's College, London, W.C.2—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 12).

Lecturer or Assistant Lecturer in Metallurgy at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, January 15).

Chair of Civil Engineering at the Imperial College of Science and Technology—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 18).

Chair of Biochemistry at the London School of Hygiene and Tropical Medicine—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 19).

Reader in Physiology at London Hospital Medical College—The Academic Registrar, University of London, Senate House, London, W.C.1 (January 26).

Reader or Senior Lecturer in the Theory of Computing in the Department of Mathematics—The Registrar, The University, Manchester 13 (January 31).

Imperial Chemical Industries Research Fellows (2) in Physics, Chemistry, Metallurgy, Engineering or Geology, or in a subject allied to these—The Registrar, University of Wales, University Registry, Cathays Park, Cardiff (March 31).

Analytical Chemist, Experimental Officer grade (preferably with a pass degree in chemistry, or at least H.N.C. (science) or equivalent, several years experience in a research or inspection laboratory, and a sound knowledge of basic analytical methods), for research and development work in an analytical laboratory—Senior Recruitment Officer, Atomic Weapons Research Establishment, Aldermaston, Berks, quoting A.629/34.

Engineer (with a first- or second-class honours degree or equivalent in engineering, and good mathematical ability) in the Structures Division of the Royal Aircraft Establishment, Farnborough, Hants, for duties which involve planning and execution of instrumented flight trials and analysis of results of such tests—The Ministry of Labour and National Service, Technical and Scientific Register (K), 26 King Street, London, S.W.1, quoting D.527/5A.

Experimental Officer or Assistant Experimental Officer (with considerable experience of the breeding and management of rabbits, rats, mice, etc., for experimental work) to take charge of the Small Animal Department—The Secretary, Rowett Research Institute, Bucksburn, Aberdeenshire.

Experimental Officer (with at least H.S.C. in science subjects, or equivalent qualifications) in the Chemical Engineering Division, for work in a cryogenic laboratory engaged on the operation of liquid hydrogen plant and the construction and operation of experimental low temperature equipment—The Establishment Officer, U.K. Atomic Energy Authority, Atomic Energy Research Establishment, Harwell, Didcot, Berks, quoting 453.

Lecturer in the Department of Electrical Engineering at the Technical College, Bradford—The Director of Education, Town Hall, Bradford.

Lecturer or Assistant Professor in Pure Mathematics, and a Lecturer or Assistant Professor in Applied Mathematics—Prof. I. R. Pounder, The University, Toronto.

Lecturers (with a good honours degree and industrial experience) in (*a*) Heat Engines, and (*b*) Fluid Mechanics, in the Department of Civil and Mechanical Engineering—The Secretary, Royal Technical College, Glasgow.

Physicists, to assist in physical and clinical work associated with X-ray and isotope treatment and investigation—The Secretary, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3.

Research Fellow in Animal Ecology for research in the ecology of the insects and mites associated with pastures in the wheat belt of South Australia—The Registrar, University of Adelaide, Adelaide, South Australia.

Senior Lecturer (with a good honours degree, together with teaching experience, and preferably with industrial and/or research experience) in Inorganic Chemistry, in the Department of Chemistry and Biology, College of Technology, Liverpool—The Director of Education, 14 Sir Thomas Street, Liverpool 1.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

University of Liverpool: Department of Social Science. Occasional Paper No. 2: Industrial Democracy—a Revaluation. By W. H. Scott. Pp. 40. (Liverpool: Liverpool University Press, 1955.) 3*s*. 6*d*. [59

Bulletin of the British Museum (Natural History). Zoology. Vol. 2, No. 13: The Morphology of the Head of the Hawfinch (Coccothraustes Coccothraustes), with special reference to the Myology of the Jaw. By Reginald William Sims. Pp. 369–393. 8*s*. Vol. 3, No. 2: The Polychaete Fauna of the Gold Coast. By Norman Tebble. Pp. 59–148+1 chart. 20*s*. Vol. 3, No. 4: A Revision of the Family Epicriidae (Acarina-Mesostigmata). By G. Owen Evans. Pp. 169–200+plates 1 and 2. 10*s*. (London: British Museum, Natural History, 1955.) [310

The Nimonic Alloys: Design Data. Third edition, extensively revised. Pp. 48. (Birmingham: Henry Wiggin and Co., Ltd., 1955.) [310

Edinburgh School of Agriculture: Edinburgh and East of Scotland College of Agriculture. Calendar for 1955–56. Pp. 84. (Edinburgh: School of Agriculture: Edinburgh and East of Scotland School of Agriculture, 1955.) [310

List of Books and Pamphlets (Supplement, concluded December, 1954) in the Special Library of the Industrial Diamond Information Bureau. Pp. 63. (London: Industrial Distributors (Sales), Ltd., 1955.) [310

Planning. Vol. 21, No. 385 (12 September, 1955): Better Industrial Design. Pp. 157–172. (London: Political and Economic Planning, 1955.) 2*s*. 6*d*. [310

The Art of Science: an Inaugural Address delivered at Makerere University College of East Africa, Kampala, Uganda, on October 28, 1954. By Prof. L. C. Beadle. Pp. iii+32. (London: Oxford University Press, 1955.) 3*s*. 6*d*. net. [310

Forestry Commission, Bulletin No. 25: Studies of North-West American Forests in Relation to Silviculture in Great Britain. By R. F. Wood. Pp. iv+43+35 photographs. (London: H. M. Stationery Office, 1955.) 6*s*. net. [310

Other Countries

Ministry of Public Works, Egypt. Nile Control Department. Paper No. 2. The Nile Basin (Fourth Supplement to Volume 4). Ten Day Mean and Monthly Mean Discharges of the Nile and its Tributaries for the years 1943–1947 and normals for the period 1912–1947. By Dr. H. E. Hurst, Y. M. Simaika and R. P. Black. Pp. viii+350. Paper No. 3: The Nile Basin (Fourth Supplement to Volume 3). Ten Day Mean and Monthly Mean Gauge Readings of the Nile and its Tributaries for the years 1943–1947 and Normals. By Dr. H. E. Hurst, Y. M. Simaika and R. P. Black. Pp. xii+359. P.T. 100; 20*s*. Paper No. 4: The Nile Basin (Second Supplement to Volume 6). Monthly and Annual Rainfall Totals and Number of Rainy Days at Stations in and near the Nile Basin and Normals for the Period 1943–1947.) By Dr. H. E. Hurst, Y. M. Simaika and R. P. Black. Pp. vii+205+4 plates. P.T. 200. (Cairo: Government Press, 1953–1955.)

Sixth Annual Report of the Commonwealth Scientific and Industrial Research Organization for the year ending 30th June, 1954. Pp. 179. (Canberra: Government Printing Office, 1954.) 10*s*. 6*d*. [59

New South Wales: Department of Agriculture. Contributions from the New South Wales National Herbarium. Vol. 2, No. 2: An Enumeration of Plants in the Albury, Holbrook and Tumbarumba Districts of New South Wales. By E. J. M. Barron. Pp. ii+89–284. (Sydney: Government Printers, 1955.) [59

New Zealand. Department of Scientific and Industrial Research: Geophysics Division. Seismological Report for October, November, December, 1952. (Bulletin, E130.) Pp. ii+20. (Wellington: Government Printer, 1955.) [59

National Physical Laboratory of India. Report 1954–55. Pp. ii+18. (New Delhi: National Physical Laboratory of India, 1955.) [59

Annales de l'Institut de Physique de Globe de l'Université de Paris et du Bureau Central de Magnétisme Terrestre. Tome 27. Pp. iv+96. (Paris: Institut de Physique du Globe du l'Université de Paris, 1954.) [59

West African Institute for Oil Palm Research. Quarterly Progress Reports. No. 13 (April 1st, 1955, to June 30th, 1955). Pp. 26. (Benin City: West African Institute for Oil Palm Research, 1955.) [310

Commonwealth Scientific and Industrial Research Organization, Melbourne. Bulletin No. 274: Sown Pastures for South-Eastern Queensland. By T. B. Paltridge. Pp. 108+6 plates. Bulletin No. 275: Common Names of Insects. Compiled under the direction of F. J. Gay. Pp. 32. (Melbourne: Commonwealth Scientific and Industrial Research Organization, 1955.) [310

Colony of Singapore. Annual Report of the Botanic Gardens Department for 1954. By J. W. Purseglove. Pp. 1+11+4 plates. (Singapore: Government Publications Bureau, 1955.) [310

Metropolitan Life Insurance Company Statistical Bulletin, Vol. 36, No. 7 (July, 1955): Mortality at New Minimum. Pp. 12. (New York: Metropolitan Life Insurance Company, 1955.) [310

Publications de l'Institut National pour l'Étude Agronomique du Congo Belge. Atlas Anatomique des Bois du Congo Belge. Vol. 1: Spermatophytes (Podocarpaceae, Cupressaceae, Ulmaceae, Moraceae, Proteaceae, Olacaceae). Par Prof. Lucien Lebacq. Pp., 58+32 plates. (Bruxelles: l'Institut National pour l'Étude Agronomique du Congo Belge, 1955.) 250 francs. [310